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THE PREPARATION OF D-α-FRUCTOHEPTOSE[1]

By R. J. Woods[2] and A. C. Neish

ABSTRACT

Crystalline D-α-fructoheptose (2-C-hydroxymethyl-D-glucose) has been obtained by the sodium amalgam reduction of D-α-fructoheptonic lactone. Some crystalline derivatives of the sugar are described and also the crystalline heptacetate of D-fructoheptitol (1,1-C-di(hydroxymethyl)-D-arabitol).

INTRODUCTION

During the course of his work upon the configuration of the sugars, Kiliani (5) condensed fructose with hydrogen cyanide and isolated a crystalline cyanhydrin which he hydrolyzed to a heptonic acid and subsequently reduced to 2-methyl hexanoic acid, thus establishing the position of the carbonyl group in fructose.

The crystalline cyanhydrin and the heptonic acid derived from it were made the subject of further research by Kiliani and Düll (2, 6, 7, 8, 9), the acid being characterized by the formation of several salts (see also 11) and a crystalline lactone, m.p. 126–130° C. Later, the acids obtained by hydrolysis of the crystalline fructose cyanhydrin and by hydrolysis of the whole of the fructose – hydrogen cyanide addition product were examined by Schmidt and Weber-Molster (12). The crystalline cyanhydrin was found to yield only the heptonic acid obtained by Kiliani while the hydrolyzate of the total addition product gave a mixture of acids, which were separated, one as the brucine salt and the other as the phenylhydrazide. The former gave a lactone identical with that obtained by Kiliani while the latter gave the epimeric acid which could be dehydrated to a crystalline anhydro lactone. The two acids were designated D-α- and D-β-fructoheptonic acid respectively, following the order of their isolation.

By comparison of the rotations shown by the free acids, the sodium salts, the amides, and the phenylhydrazides, Schmidt and Weber-Molster showed that the *alpha* and *beta* acids behaved similarly to gluconic and mannonic acids respectively and on this basis suggested the following structures:
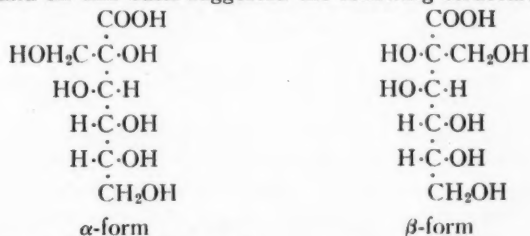
COOH	COOH
HOH₂C·C·OH	HO·C·CH₂OH
HO·C·H	HO·C·H
H·C·OH	H·C·OH
H·C·OH	H·C·OH
CH₂OH	CH₂OH
α-form	β-form

If these structures are correct the sugar obtained by reduction of the alpha lactone is 2-C-hydroxymethyl-D-glucose; the trivial name D-α-fructoheptose is used in this paper.

Fischer (3) noted in an early paper on the sodium amalgam reduction of sugar lactones that fructoheptonic lactone could be reduced to a sugar by this method, though he isolated no pure products. He stated that further work was in progress but we have not been able to find any subsequent references to the preparation of D-α-fructoheptose. We have repeated the reduction of D-α-fructoheptonic lactone with sodium amalgam to give solutions containing D-α-fructoheptose in up to 65% of the theoretical yield.

In the first instance the sugar was obtained as a syrup or amorphous, very deliquescent, solid from which it was very difficult to isolate any pure derivative. However, 2:5-dichlorophenylhydrazine was found to give a hydrazone that formed readily and was easily purified. Regeneration from this derivative led to a sugar syrup that finally crystallized, subsequent reductions yielding the crystalline sugar directly on seeding. The crystalline sugar was stable and nondeliquescent. Neither the α-fructoheptonic lactone nor the sugar itself showed any evidence of anhydride formation as described for the β-lactone. Assuming aqueous D-α-fructoheptose solutions to mutarotate similarly to the straight chain sugars, the change in rotation to give a less dextrorotatory solution indicated that the crystalline sugar isolated was an α-D-anomer (4). No evidence concerning the ring size was obtained.

The sugar was catalytically hydrogenated to give the corresponding sugar alcohol, isolated as the crystalline heptacetate. The free alcohol has not yet crystallized.

EXPERIMENTAL

Melting points were determined on a heating stage microscope and, unless otherwise stated, are corrected.

Fructose Cyanhydrin

The method of Zervas and Sessler (16) was followed. It proceeded smoothly once seeds of the cyanhydrin had been obtained to promote crystallization of the condensation product. The fructose cyanhydrin was washed with cold methanol and dried in a vacuum desiccator for 18–20 hr. over calcium chloride before being hydrolyzed. It was obtained as a white crystalline solid, m.p. 108–109° C. (uncorr.), in yields of 50 to 65%; usually one mole was prepared at a time.

D-α-Fructoheptonic Lactone

The cyanhydrin was hydrolyzed by hydrochloric acid and the product worked up essentially as described previously (8, 12). Removal of the hydrogen chloride was greatly expedited by use of an evaporator similar to that described by Bartholomew (1), arranged to recycle automatically. Remaining inorganic materials were removed as previously described (12) and final traces of cations by passage through a column of ion-exchange resin (Amberlite IR-120). Yields of heptonic acid of 85–90% (by titration) were obtained with

a hydrolysis period of two and one-half hours and yields of 68–78% with a hydrolysis period of four hours. A purified acid was obtained, by regeneration from the once-recrystallized brucine salt, and lactonized by heating to 130° C. for five hours. The syrup crystallized on cooling to give the lactone, m.p. 118–119° C. (uncorr.) in 57% over-all yield (two and one-half hours hydrolysis). One crystallization from ethanol raised the melting point to 129.5° C., unchanged by further crystallization.

Reduction D-α-Fructoheptonic Lactone

A solution of sodium hydrogen oxalate was prepared by dissolving oxalic acid dihydrate (126 gm.) in warm water (1100 ml.) and adding sodium hydroxide solution (100 ml.; 10 N). D-α-Fructoheptonic lactone (180 gm.; m.p. 118–119° C.) was added and the solution cooled to 5° C. Sodium amalgam (2800 gm.; 3%) was stirred into the solution in four equal portions, keeping the temperature below 15° C. and keeping the pH below 4.0 by the addition of powdered oxalic acid. The mixture was then stirred for 45 min. and filtered while still cold. The solid was washed with a little cold water and the filtrate treated with an excess of calcium acetate, the precipitated calcium oxalate being filtered off and the filtrate passed through a column of cation exchange resin (Amberlite IR-120). The amount of aldose present in the solution, estimated by the reduction of hypoiodite, corresponded to a 65% yield of the required sugar. The solution was concentrated under reduced pressure.

The crude reduction mixture could not be crystallized and did not form any derivatives readily. Phenylhydrazine and as-diphenylhydrazine did not give solid hydrazones although a crystalline derivative was later obtained with 2: 5-dichlorophenylhydrazine.

Reduction of D-α-fructoheptonic lactone with sodium borohydride by a method similar to that described by Wolfrom and Wood (14), but in the presence of a sodium hydrogen oxalate buffer, gave a solution containing 49% of the theoretical quantity of aldose, estimated by hypoiodite. Since this reagent appeared to offer no advantage over the use of sodium amalgam the reduction employing it was not studied further.

D-α-Fructoheptose 2,5-Dichlorophenylhydrazone

The syrupy D-α-fructoheptose obtained above was dissolved in methanol (750 ml.) (cf. Mandl and Neuberg (10)) and treated with an excess (15%) of 2,5-dichlorophenylhydrazine. The crude derivative was washed with ethanol and water and crystallized once from aqueous pyridine (201 gm., m.p. 188° C., uncorr.), further crystallization gave the pure *hydrazone* as white plates, m.p. 190° C. Found: C, 42.45; H, 5.05; Cl, 19.25. $C_{13}H_{18}O_6N_2Cl_2$ requires: C, 42.3; H, 4.9; Cl, 19.2%, $[\alpha]_D^{22.2°}$ 7.9° (c, 3.6 in pyridine) changing to $[\alpha]_D^{22.3°}$ 5.05°.

D-α-Fructoheptose

A solution of the sugar 2,5-dichlorophenylhydrazone (178 gm.; obs. m.p. 188°) in ethanol (1500 ml.) and water (2500 ml.) was refluxed with benzaldehyde (500 ml.) and benzoic acid (50 gm.) for 18 hr. (cf. Sowden and Fischer (13)). After cooling, the aqueous layer was separated and thoroughly washed

with chloroform and ether and evaporated to give the sugar as a pale yellow syrup (79 gm.), which crystallized from an ethanolic solution after several weeks as needles (70.5 gm.), m.p. 171.5° C. A sample was recrystallized by dissolving it in a minimum of water and adding glacial acetic acid or ethanol (about five volumes). The pure *sugar* separated slowly as sweet white needles, very soluble in water, m.p. 172.5–173.5° C. Found: C, 40.0; H, 6.7. $C_7H_{14}O_7$ requires: C, 40.0; H, 6.7%, $[\alpha]_D^{18.2°}$ 51.9° (*c*, 5 in water) mutarotating to $[\alpha]_D^{20.5°}$ 42.95°.

In later experiments, in which seed was available, the sugar could be crystallized directly from the syrupy reduction product by diluting with several volumes of glacial acetic acid and seeding. About 80% of the sugar present crystallized directly and the greater part of the remainder could be recovered by treating the mother liquors with 2,5-dichlorophenylhydrazine.

An attempt to regenerate the sugar by treating the hydrazone above with acetaldehyde, as suggested by Mandl and Neuberg (10), was unsuccessful and neither the sugar nor the hydrazone could be recovered.

Other Hydrazones of D-α-Fructoheptose

The pure sugar formed a *phenylhydrazone*, obtained as unstable, transparent plates from methanol, m.p. 144.5°. Found: C, 52.2; H, 6.75; N, 9.4. $C_{13}H_{20}O_6N_2$ requires: C, 52.0; H, 6.7; N, 9.35%, $[\alpha]_D^{22.2°}$ 9.81° (*c*, 1.6 in ethanol) changing to $[\alpha]_D^{22.2°}$ 1.46° after 40 hr. No osazone could be detected under the usual conditions of osazone formation. The *p-nitrophenylhydrazone* crystallized from water as golden plates and needles, discoloring slightly in light, m.p. 178.5° C. Found: C, 45.3; H, 5.5; N, 12.10. $C_{13}H_{19}O_8N_3$ requires: C, 45.2; H, 5.55; N, 12.15%, $[\alpha]_D^{22.2°}$ −26.3° ± 0.8° (*c*, 0.5 in water) changing to $[\alpha]_D^{21.5}$ −21.6° ± 0.4° after 95 hr. The melting point fell to 150° when the derivative was stored in the dark for six weeks. Though the phenylhydrazone and *p*-nitrophenylhydrazone decomposed on keeping the 2,5-dichlorophenylhydrazone appeared to be quite stable.

D-*Fructoheptitol Heptacetate*

Crystalline D-α-fructoheptose (20 gm.) in water (60 ml.) was shaken in an atmosphere of hydrogen in the presence of a Raney nickel catalyst (1–2 gm.) until the uptake of hydrogen ceased (four hours at 100° C. and 2500 lb./sq. in.). The catalyst was filtered off and the filtrate concentrated to give a pale yellow syrup (21.7 gm.) containing less than 5% aldose (estimated by hypoiodite).

A portion of the syrupy alcohol (13.3 gm.) was refluxed with anhydrous sodium acetate (12 gm.) and acetic anhydride (150 ml.) for three and one-half hours. The gummy acetate, isolated by chloroform extraction, was dissolved in benzene (30 ml.) and filtered through a column of activated aluminum oxide (400 gm.; Merck, washed with 10% acetic acid and water and dried at 180°) and eluted with the same solvent. Fractions (50 ml.) were collected and evaporated, those constituting the main band solidified and were crystallized from ethanol to give D-*fructoheptitol heptacetate* as white needles (24.8 gm.) m.p. 70.5° C. Found: C, 49.85; H, 6.05; $CH_3.CO$, 59.25. $C_{21}H_{30}O_{14}$ requires: C, 49.8; H, 5.95; $CH_3.CO$, 59.5%, $[\alpha]_D^{24.2}$ 31.6° (*c*, 7 in chloroform). The chro-

matographic purification could be omitted in later preparations in which the crude acetate solidified on seeding.

Acetylation in pyridine solution gave a mixture of products from which the heptacetate could be isolated by chromatography, though in poorer yield than described above.

D-*Fructoheptitol* [1,1-C-di(*hydroxymethyl*)-D-*arabitol*]

The alcohol was regenerated from the heptacetate (15.0 gm.) by solution in anhydrous methanol (70 ml.) containing a little sodium (0.05 gm.) (15). The solution was evaporated under reduced pressure after standing at room temperature (23° C.) overnight and yielded a colorless syrup (7.23 gm.), $[\alpha]_D^{21.8}$ −5.69° (c, 11 in water).

ACKNOWLEDGMENTS

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THE SYNTHESIS OF SYRINGALDEHYDE FROM VANILLIN[1]

By J. M. PEPPER AND J. A. MacDONALD

ABSTRACT

Syringaldehyde has been synthesized in high yields from vanillin. The process consists of the iodination of vanillin, followed by the interaction of the resultant 5-iodovanillin with sodium methoxide in anhydrous methanol at temperatures of $130 \pm 4°$ C. for one hour in the presence of a copper catalyst. Along with the syringaldehyde, small amounts of unchanged 5-iodovanillin and vanillin were always found in the reaction mixture. Analysis of the final product was made by an initial separation of the components by downward paper chromatography using a mixture of petroleum ether (b.p. 100–120° C.), di-n-butyl ether, and water (10:1:1) as the developing agent for a period of 10 hr. The separated compounds were extracted from the paper and their concentrations in alcoholic alkaline solutions determined spectrophotometrically.

Under conditions by which 5-iodovanillin was converted to syringaldehyde in better than a 95% yield, 5-bromovanillin gave only a 61% yield and 5-chlorovanillin gave no detectable amounts of syringaldehyde.

In connection with an investigation into the fundamental nature of isolated lignins and their degradation products from angiosperms (poplar wood and cereal straws), it was important to have available many reference compounds of known structure. For such work the compounds required are, for the most part, of two types: those containing the guaiacyl (4-hydroxy-3-methoxyphenyl) nucleus and those containing the syringyl (4-hydroxy-3,5-dimethoxyphenyl) nucleus. Many derivatives having the former structure have been synthesized from vanillin (4-hydroxy-3-methoxybenzaldehyde) which is readily available as an oxidation product of gymnosperm lignin or its derivatives. Much less work has been reported on the chemistry of compounds having the syringyl nucleus. A logical starting material for their syntheses would be the corresponding aldehyde, syringaldehyde (4-hydroxy-3,5-dimethoxybenzaldehyde). Even though the oxidation of lignified angiosperm material gives rise to a mixture of both vanillin and syringaldehyde, the separation and recovery of each is not readily accomplished, so another source of this latter aldehyde is preferred.

Many syntheses, as outlined by Pearl (9), have been reported, and a laboratory procedure has appeared in Organic Syntheses (1). The main starting material in these procedures is either 1,3-dimethylpyrogallol or gallic acid, the conversion of either of which to the aldehyde involves many separate steps and therefore low over-all yields. A recent publication (10) describes a new synthesis involving the conversion of vanillin to 5-hydroxyvanillin, complete methylation followed by selective demethylation of the 4-methoxy group to give syringaldehyde in good yield. These authors also report that their attempts at the conversion of 5-halovanillin to syringaldehyde were universally unsuccessful. Some time ago an investigation was begun in these laboratories to study this same synthesis of syringaldehyde from vanillin by the introduction

of a methoxy grouping. One value of such a method would be the possibility of thereby converting a mixture of the aldehydes obtained by the oxidation of hardwoods into pure syringaldehyde should a valuable use be found for this chemical.

In a previous communication (8) it was reported that preliminary experiments on the two-step synthesis: vanillin → 5-bromovanillin → syringaldehyde, appeared promising. By treatment of the 5-bromovanillin with sodium methoxide in anhydrous methanol in the presence of copper turnings at temperatures of 160–200° C., small yields of syringaldehyde were obtained along with other reaction products. Because of the well known reactivity of halogen substituted aromatic compounds increasing from chloro- through bromo- to iodo-derivatives in nucleophilic substitution reactions (3), it was thought that the use of 5-iodovanillin in a similar synthesis would be more suitable. Excellent yields of syringaldehyde (II) have now been obtained from vanillin (I) by this method according to the following equations:

I   II
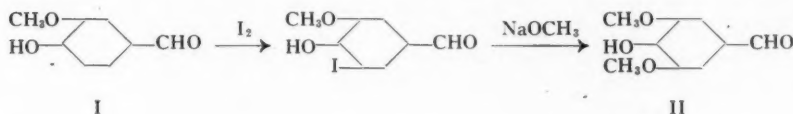
Preliminary runs were carried out in which 5-iodovanillin, anhydrous methanol, sodium methoxide, and a copper catalyst were heated together in a rocking autoclave at temperatures ranging from 72° C. to 180° C. and for reaction times of from one to eight hours. After dilution of the orange to dark red reaction mixture with water and filtration to remove the copper, the products were extracted into chloroform and then treated as follows. First they were separated into bicarbonate-soluble and bicarbonate-insoluble fractions, and then each of these was subdivided into sodium bisulphite-soluble and sodium bisulphite-insoluble portions. It was often noticed that complete acidification of the original alkaline reaction mixture caused any unchanged 5-iodovanillin to precipitate. This insolubility of 5-iodovanillin in acidic aqueous methanol permitted its ready separation from the other components of the reaction mixture. Support was found in these experiments for an observation reported earlier (11) that, from "neutral" lignin fractions, compounds having a syringyl nucleus were isolated. In this case the incomplete liberation of phenolic compounds by the exhaustive carbonation of a strongly alkaline solution was borne out by finding phenolic aldehydic products in both the bicarbonate-soluble and bicarbonate-insoluble portions.

The aqueous residues from the various runs were analyzed for iodide ion by a standard method (12). These results along with the amount of unchanged 5-iodovanillin recovered are given in Table I, where iodide ion is expressed as the corresponding weight of 5-iodovanillin. The values reported for the iodide ion determinations are averages of duplicate analyses which agreed within one per cent and are minimum values since, prior to the analyses, the aqueous solutions had been acidified and extracted several times with chloroform. It

TABLE I

Preliminary experiments using 5-iodovanillin[a]

Run No.	Average temp., °C.	Reaction time, hr.	Nonaldehydic fraction[b], gm.	Syringaldehyde containing fraction[c], gm.	Percentage of original 5-iodovanillin		
					Recovered unchanged	Converted to iodide	Total accounted for
1	179	1.0	8.8	2.0	Nil	—	—
2	154	5.0	7.1	0.8	Nil	96.0	96.0
3[d]	72	7.5	0.3	0.6	82.4	7.8	90.2
4	137	1.25	3.9	3.9	Nil	93.4	93.4
5	110	1.0	—	4.8	36.9	48.3	85.2
6	123	1.2	0.3	6.2	19.2	77.6	96.8
7	118	1.0	1.0	4.9	25.4	68.0	93.4
8	118	6.25	—	7.2	Nil	92.8	92.8
9	123	8.5	3.0	6.0	Nil	93.4	93.4
10	116	8.0	0.4	4.3	13.0	81.0	94.0

[a] For each run, 5-iodovanillin (13.0 gm.), anhydrous methanol (250 ml.), sodium (10.0 gm.) (with the exception of Run 1 in which 5.0 gm. were used), and copper catalyst A (12.8 gm.) were reacted.
[b] Bicarbonate-insoluble, sodium bisulphite-insoluble.
[c] Combined bicarbonate-insoluble, sodium bisulphite-soluble, and bicarbonate-soluble fractions.
[d] Run carried out at atmospheric pressure under reflux.

seems reasonable to conclude that, under the conditions employed, all or nearly all of the 5-iodovanillin which reacts loses iodine.

Varying small amounts of syringaldehyde were isolated by crystallization and sublimation from many of the fractions obtained in these preliminary experiments, but the amounts given in Table I represent only the weight of the crude products shown to contain syringaldehyde. It appears that, with such a reaction mixture, increasing temperatures lead to an increase in the syringaldehyde-containing fractions until a temperature of 135° C. is reached and that above this temperature the yield of the nonaldehydic fraction becomes more and more appreciable. The chemical nature of this derivative, referred to as the "Unknown" in Fig. 1, has not as yet been determined but qualitative tests show that it is phenolic and contains no iodine.

In an analysis of the reaction product of Run 10 by a chromatographic method, an appreciable quantity of vanillin (28%, based on 5-iodovanillin) along with syringaldehyde was detected. The presence of vanillin, characterized by the comparison of its ultraviolet absorption spectrum and chromatographic behavior with an authentic sample, is not surprising. The reductive cleavage of aryl halides is not uncommon under similar alkaline conditions (3, 10).

The lack of a single fraction containing all of the syringaldehyde, and the detection of vanillin in the reaction product, indicated that a new method of determining yields was required. A method of analysis similar to that used by Stone and Blundell (13) proved satisfactory. The use of a 10:1:1 mixture of petroleum ether (b.p. 100–120° C.), di-n-butyl ether, and water as the developing solvent gave an adequate separation of syringaldehyde, vanillin, 5-iodovanillin, and the unidentified reaction product on a paper strip (Fig. 1).

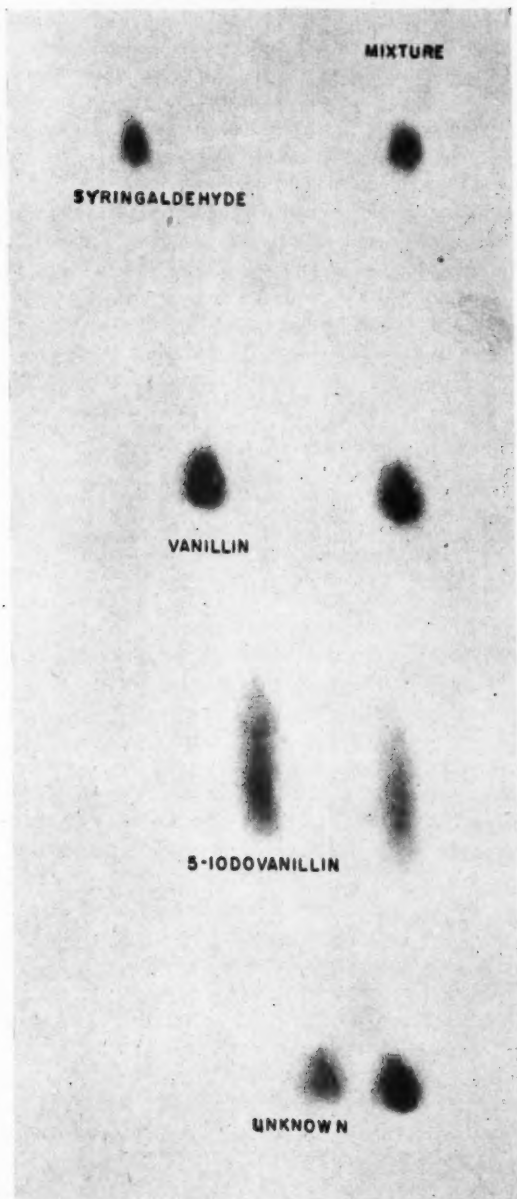The positions of the various phenolic compounds were determined by

FIG. 1. Chromatographic separation of syringaldehyde, vanillin, 5-iodovanillin, and an unidentified phenolic reaction product. Chromatogram developed for 10 hr. using petroleum ether (b.p. 100–120° C.), butyl ether, and water (10.1.1).

spraying test strips of the chromatogram with a ferric chloride – potassium ferricyanide mixture (2). The separated bands were then extracted with ethanol and the concentrations of the extracts determined spectrophotometrically. Such analyses of known mixtures showed that an average of 7.6% of the syringaldehyde, 18.0% of the vanillin, and a negligible amount of 5-iodovanillin were lost during the analysis. The results of subsequent analyses were corrected for these losses.

A second series of experiments was then carried out and the products analyzed by this preferred method. The results are given in Table II. The results of Runs 11 and 12 indicate that a copper catalyst is necessary in this reaction. It was thought that the extreme variations in yields of products of Runs 11, 13, 14, and 15 were not due primarily to the slight temperature differences recorded but rather to differences in the catalyst, a fresh batch of which had

TABLE II

CONVERSION OF 5-IODOVANILLIN TO SYRINGALDEHYDE[a]

Run No.	Copper catalyst[b]	Average temp., °C.[c]	Analysis of reaction mixture			
			Component[d]	Duplicate analyses, gm.		Mean yield, %	Total yield, %
11	A	122	SA	5.39	5.38	63.2	
			V	1.30	1.26	18.0	
			5IV	2.35	2.40	18.3	99.5
12	—	122	SA	0.52	0.52	6.1	
			5IV	12.7	12.3	96.3	102.4
13	A	128	SA	7.50	7.32	87.0	
			V	0.28	0.25	3.7	
			5IV	1.61	1.64	12.5	103.2
14	A	133	SA	6.38	6.59	76.2	
			V	0.88	0.92	12.7	
			5IV	1.39	1.30	10.4	99.3
15	A	128	SA	7.10	7.02	83.0	
			V	0.92	0.98	13.4	
			5IV	0.18	0.12	1.5	97.9
16	B	126	SA	8.42	8.44	98.9	
			V	0.14	0.18	2.2	
			5IV	0.31	0.33	2.5	103.5
17	B	129	SA	8.41	8.26	97.9	
			V	0.20	0.20	2.8	
			5IV	0.25	0.25	1.9	102.6

[a]　*For each run 5-iodovanillin (13.0 gm.), sodium (10 gm.), anhydrous methanol (250 ml.), and copper catalyst (12.8 gm.*) were used; reaction time one hour at elevated temperature.*
[b]　*Catalyst A prepared by precipitating copper from copper sulphate solution with zinc; catalyst B was British Drug Houses' precipitated copper powder, reagent grade.*
[c]　*Maximum temperature variation, ±4° C.*
[d]　*Abbreviations: SA for syringaldehyde, V for vanillin, and 5IV for 5-iodovanillin.*

*　*Some later work has indicated that under similar conditions this amount of copper may be reduced to 2.0 gm. without serious decrease in percentage conversions and also that yields of around 50% of syringaldehyde may be obtained if 0.5 gm. iodine are used in place of the metallic copper catalyst.*

been prepared for each run. Using catalyst portions taken from the same source (Catalyst B), reproducible results were obtained (Runs 16 and 17). Furthermore, the high yields of syringaldehyde obtained indicated that the British Drug Houses precipitated copper powder was the preferred catalyst for this reaction. Subsequently, many runs similar to Runs 16 have been made and pure syringaldehyde readily recovered and recrystallized as light yellow needles from Skellysolve "C", m.p. 109–110° C. in yields of 85% or better.

The possibility of converting either of the cheaper 5-chloro- or 5-bromo-vanillin to syringaldehyde under similar conditions was investigated. Both these 5-halovanillins moved further than vanillin or syringaldehyde under the previously used chromatographic conditions and hence did not interfere with the analyses. Using 5-bromovanillin, runs carried out at 130° C. and 140° C. gave rise in each case to products representing a 61% conversion to syringaldehyde. Under similar conditions using 5-chlorovanillin at any of 130° C., 140° C., or 175° C., no syringaldehyde was detected. This decreased conversion of the 5-halovanillins to syringaldehyde from 5-iodo- through 5-bromo- to 5-chloro- is in agreement with the known reactivities of halogen substituted aromatic compounds.

This method for the preparation of large amounts of syringaldehyde has since been used very successfully in our laboratories.

EXPERIMENTAL*

Reagents.—The 5-halovanillins were prepared according to the following previously reported procedures: 5-iodovanillin (m.p. 178.5–179.5° C.) by the method of Erdtman (6)**; 5-bromovanillin (m.p. 164° C.) by McIvor and Pepper (8), and 5-chlorovanillin (m.p. 160.5–162° C.) according to Hopkins and Chisholm (7).

Copper Catalyst A.—The early experiments were carried out using a copper preparation prepared by the addition of zinc to copper sulphate solution according to the method of Brewster and Groening (4). The catalyst was prepared immediately prior to each run and washed with 10 portions of absolute methanol before use.

Copper Catalyst B.—British Drug Houses' precipitated copper powder, Reagent Grade, was used in the later experiments.

Apparatus.—All the reactions were carried out in a stainless steel liner, capacity 1080 ml., of an Aminco high pressure hydrogenator, Model No. 406-01 DA. This apparatus served as a rocking autoclave. A Brown Indicating Controller (0–600° C.) coupled with an Aminco variable voltage transformer inserted into the automatic heating circuit permitted temperature control to within ±4° C. A Beckmann Model DU spectrophotometer was used to obtain the ultraviolet absorption data.

Procedure Employed in Runs 11–17

These runs (Table II) were maintained at the stated reaction temperatures for one hour, after which the bomb was cooled slowly. In each case the product

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* *All melting points are corrected.*
** *It appears that the 0.2 N sodium hydroxide reported should read 2.0 N sodium hydroxide.*

was diluted with water (500 ml.) and filtered to remove the copper. The filtrate, which varied in color from yellow to orange red, was analyzed by the chromatographic–spectrophotometric method.

The chromatography was carried out according to the method of Stone and Blundell (13). Strips of Whatman No. 1 filter paper, 22 in. × 6 in., were spotted along a base line with the alkaline reaction mixture. The volumes of the drops were measured by a 1 ml. capacity Emil Greiner ultramicroburette which was graduated in 0.001 ml. divisions. After acidification of the material on the paper using acetic acid vapor, the development was conducted in a descending manner in an apparatus similar to that described by Consden *et al.* (5). Water was placed in the bottom of a tall glass cylinder and the organic phase from a 10: 1: 1 mixture of petroleum ether (b.p. 100–120° C.), di-*n*-butyl ether, and water was placed in the small trough near the top and also in a small beaker placed on the bottom of the tightly closed vessel which was allowed to stand for one-half hour prior to putting the paper in place. After development for 10 hr., the paper strips were removed, and the test strip cut off and sprayed with a 1% solution of ferric chloride followed by a 1% solution of potassium ferricyanide, which indicated, by pronounced blue spots, the position of the phenolic compounds (2). The main chromatogram was then cut into horizontal strips, each of which contained only one compound which was separately extracted with ethanol in Soxhlet extractors for two hours. Each extract was transferred to a 50 ml. volumetric flask containing 4 ml. of 0.2 N potassium hydroxide in ethanol, and made up to 50 ml. with ethanol.
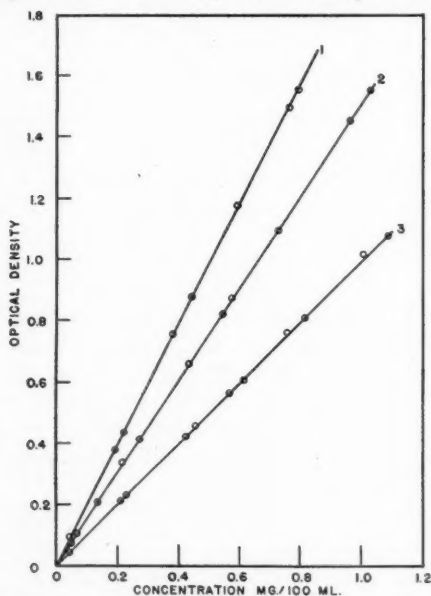

FIG. 2.   Concentration vs. optical density curves in alkali-ethanol for (1) vanillin, (2) syring-aldehyde, and (3) 5-iodovanillin.

The concentration of the extract was then determined by the measurement of its optical density and by making use of previously determined optical density versus concentration curves (Fig. 2). The wave lengths used were those determined by an examination of the ultraviolet absorption spectra of the compounds in alcoholic potassium hydroxide and were 368, 352, and 356 mμ for syringaldehyde, vanillin, and 5-iodovanillin, respectively.

Syringaldehyde.—Run 16 serves to illustrate a typical preparation of syringaldehyde by this method. Sodium (10.0 gm.) was dissolved in anhydrous methanol (250 ml.). This solution together with 5-iodovanillin (13.0 gm.) and British Drug Houses' precipitated copper powder catalyst (12.8 gm.) was heated in the rocking autoclave at 124–128° C. for one hour. After cooling, the reaction product was diluted with water (500 ml.), filtered to remove the copper, and acidified with hydrochloric acid. A small amount of unchanged 5-iodovanillin precipitated and was removed by filtration. The filtrate was extracted with chloroform (5 × 200 ml.) and the chloroform extract back extracted first with a few milliliters of sodium thiosulphate, to remove any free iodine, and then with water (2 × 10 ml.). After drying over anhydrous sodium sulphate the chloroform solution was concentrated to a small volume by distillation. The residue was crystallized from Skellysolve "C", yielding light yellow needles (7.28 gm.) (85.5%), m.p. 109–110° C. A mixed melting point with authentic syringaldehyde prepared according to Pearl (9) showed no depression. A similarly obtained product, recrystallized several times from Skellysolve "C" and twice from water, melted at 109.8–110.2° C. Calc. for $C_9H_{10}O_4$: C, 59.32; H, 5.53%. Found*: C, 59.33, 59.20; H, 5.59, 5.55%.

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* *Analyses by Dr. R. U. Lemieux, National Research Council Laboratory, Saskatoon, Sask.*

THE EFFECT OF RELATIVE HUMIDITY ON THE REACTION OF NINHYDRIN WITH AMINO ACIDS ON PAPER CHROMATOGRAMS[1]

By Eunice F. Wellington[2]

ABSTRACT

The effect of different relative humidities at 20° C. on the colored product of ninhydrin with amino acids on paper chromatograms has been investigated. It has been shown that there is only a very narrow range of humidity (35–40%) within which maximum color development for most amino acids can be expected (aspartic acid behaves anomalously), and that the color intensity falls off rapidly on both sides of this maximum.

INTRODUCTION

Earlier workers (2) attempting to use paper chromatography as a quantitative tool for analysis of amino acids by the ninhydrin reaction found that there was considerable day-to-day variability in color yields. This variability was assumed to result from changing temperature and humidity, although no definite tests of this assumption were made.

Later, Thompson *et al.* (3) showed that a constant temperature was necessary for reproducible results. They also controlled the humidity by developing the color in special tanks containing an atmosphere of carbon dioxide and ethyl alcohol. However, they reported no experiments on the direct effect of relative humidity on the reaction of amino acids with ninhydrin.

Wellington (4) recently showed that reproducible results could be obtained by a simpler technique than that of Thompson *et al.* The technique required that the papers be allowed to stand for 30 hr. at $20 \pm 1°$ C. and $40 \pm 3\%$ relative humidity after having been sprayed with ninhydrin. Since air-conditioning equipment is not available in most laboratories however, the effect of humidity on the color yield of the reaction of amino acids with ninhydrin has been evaluated more critically, and a simple apparatus affording the necessary degree of control has been developed.

MATERIALS AND METHODS

Two-dimensional paper chromatograms of standard amounts of amino acids were carried through according to the method previously described (4), with the exception that different levels of relative humidity were tested during color development. The sprayed chromatograms were hung in separate cylinders, in each of which the atmosphere was held for 30 hr. at a constant relative humidity by means of a glycerine–water mixture in the bottom of the cylinder. Layering of the atmosphere above these mixtures was prevented by passing a rapid stream of compressed air into the cylinders through gas-washing bottles filled with corresponding glycerine–water mixtures. Thus, the air was kept at a constant humidity throughout each tank.

The glycerine–water mixtures were prepared according to Carson's table (1). Since the table was prepared for glycerine–water mixtures at 25° C., and the present work was done at 20° C., the relative humidity actually obtained with each of these mixtures was measured during the tests with paired wet and dry thermocouples and a potentiometer.

RESULTS AND DISCUSSION

The following amino acids were tested: cysteic acid, glutamic acid, aspartic acid, serine, glycine, threonine, alanine, valine, leucine, histidine, arginine, and lysine. All these except aspartic acid were affected in the manner illustrated by glutamic acid, serine, and lysine (Fig. 1) with maximum development
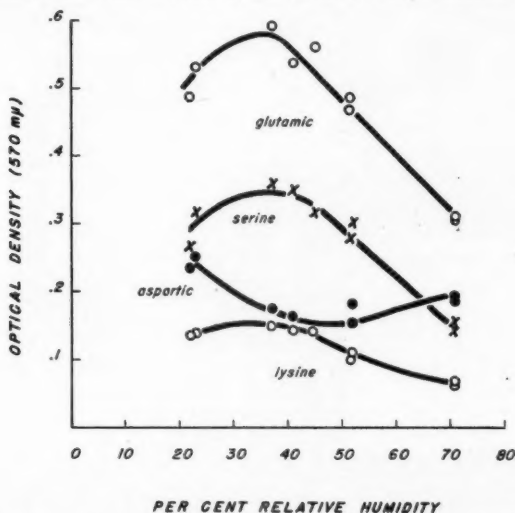
FIG. 1. The effect of relative humidity at 20° C. on the intensity of the colored product formed by the reaction of amino acids with ninhydrin on paper chromatograms (glutamic acid 26γ, serine 13γ, aspartic acid 23γ, and lysine 8γ).

of the colored ninhydrin product in the range of 35–40% relative humidity. Aspartic acid behaves in an anomalous manner, giving a minimum yield of the colored compound near the range where the other amino acids give a maximum.

It is obvious that variations in relative humidity between 40 and 70%, a condition common in laboratories not equipped with air-conditioning, are sufficient to explain the day to day variability in the ninhydrin reaction reported by earlier workers. This variability, together with the fact that earlier workers were using a spray containing 1/5 to 1/20 the concentration of ninhydrin required to give maximum color yields (4), made quantitative determinations of amino acids on paper chromatograms practically impossible.

These results confirm the necessity for the rigid control of humidity previously emphasized (4). In laboratories without air-conditioning, the chromatograms may be hung in a box through which compressed air is blown. The

compressed air should be brought to at least 35% R.H. by bubbling it from a sintered glass filter through a 60-cm. column of glycerine–water mixture (specific gravity 1.234–1.235). Pure glycerine*–water mixture is particularly suitable since, in contrast with some of the salt and acid solutions tried for this purpose, it has no effect on the development of the ninhydrin color. Furthermore, if the compressed air is originally drier than 35% R.H., only small amounts of water have to be added to the mixture after each run to bring it back to correct proportions.

Fig. 2 shows the details of the apparatus used. The compressed air is passed
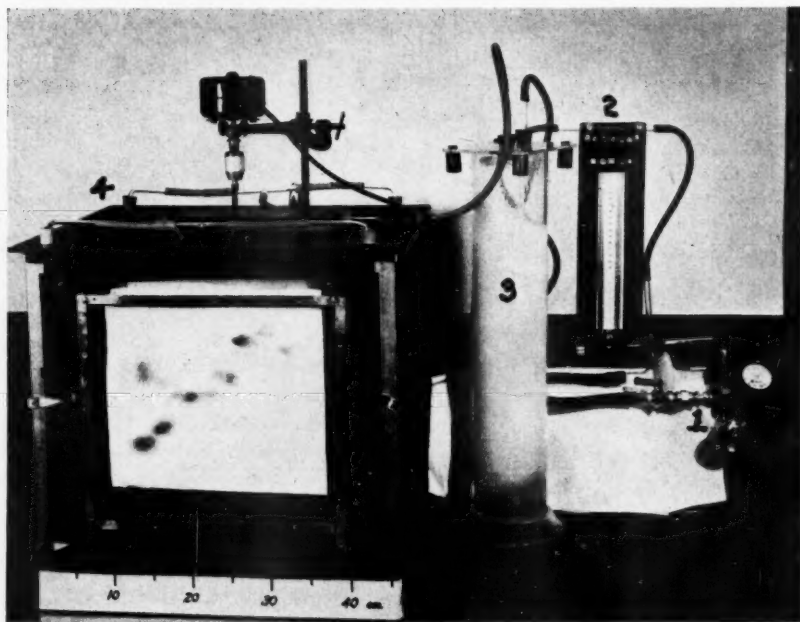
FIG. 2. Apparatus for the color development of amino acids on paper chromatograms under conditions of controlled relative humidity:

        1—compressed air source with reducing valve;
        2—flowmeter;
        3—glycerine–water mixture to control relative humidity;
        4—development box, black front cover removed to show interior.

through the first sintered-glass filter to remove any dirt particles and then it passes over an air flow gauge (flow regulated to approximately 8 liters per min.) through a second filter into the glycerine–water mixture. The conditioned air then passes into a 45 cm. square blackened glass box through inlets at each corner, and a 20 cm. fan underneath the papers keeps the air in the box well mixed. A light metal frame inside the box holds 10 papers, each 25.5 × 27.5 cm.

With such an apparatus, the relative humidity can easily be held within ±1%. Furthermore, undesirable fumes are more easily kept away from the

    * *Some batches of reagent glycerine are not satisfactory for this purpose since they contain impurities that cause the mixture to foam badly.*

papers. (As previously mentioned (4), exposure of the papers to even small amounts of hydrochloric acid fumes between the completion of the second chromatographic run and the elution of the colored ninhydrin product will cause anomalous results. Extremely small amounts of this vapor produce variations in color intensity, larger amounts cause the color to change from purple to pink, and still larger amounts prevent the development of the color entirely.)

Previous work (4) showed that it was essential that relative humidity be kept constant for repeatable quantitative results. The present results demonstrate for the first time that there is only a very narrow range of humidity within which maximum color development for most amino acids can be expected, and that the color intensity falls off rapidly on both sides of this maximum.

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THE BIOGENESIS OF ALKALOIDS

VIII. THE ROLE OF METHIONINE IN THE FORMATION OF THE N-METHYL GROUPS OF THE ALKALOID HORDENINE[1]

BY T. J. MATCHETT,[2] LÉO MARION, AND SAM KIRKWOOD[2]

ABSTRACT

L-Methionine labelled with C^{14} in the methyl group was fed to sprouting barley and hordenine and choline were isolated from the plants. These two molecules proved to be labelled solely in their N-methyl groups. A comparison of formate and methionine methyl as precursors of hordenine methyl shows that the latter was more efficient. The fact that choline methyl does not serve as a precursor of hordenine methyl was confirmed and it was shown that bicarbonate is also ineffective as a methyl precursor. The significance of these findings to the over-all methylation process of the plant is discussed.

Recently the labile methyl metabolism of the higher plants has come under scrutiny in connection with investigations into the origin of the N- and O-methyl groups of the plant alkaloids. It has been shown that formate carbon serves as a precursor of the methyl groups of both hordenine and choline in the barley plant (8). Since choline did not serve as a methyl donor in the formation of hordenine methyl, it was assumed that these methyls did not arise through a process of transmethylation but were produced by a formylation reaction followed by a reduction. This reasoning was based on the fact that in animal metabolism there is a reversible transfer of methyl between choline and methionine. If this reaction occurred in the barley plant, both choline and methionine should have been labelled when labelled choline was fed. Since under these conditions the hordenine methyl was not labelled it was assumed that it did not arise as a result of a transmethylation reaction. Subsequently it was shown by Brown and Byerrum (2) that the methyl carbon of methionine and formate carbon serve as precursors of the N-methyl group of the alkaloid nicotine and that the former is the more efficient precursor. Brown and Byerrum postulate that methionine methyl is transmethylated to nicotine and that formate serves as a precursor of methionine methyl. Dubeck and Kirkwood (3) have shown that methionine methyl serves as a precursor of both the N- and O-methyl groups of the alkaloid ricinine which is synthesized by germinating beans of *Ricinus communis*. In this case both formate and choline methyl failed to serve as precursors of these methyl groups. All of this evidence focuses attention on the central role of methionine in processes involving labile methyl. In view of this we have investigated the role of methionine in the origin of hordenine methyl. We have also reinvestigated the failure of choline to serve as a precursor of hordenine methyl.

EXPERIMENTAL

Measurement of Radioactivity

All radioactive compounds were measured in a "Q"-gas counter and appropriate corrections were made.

Labelled Compounds

C^{14}-methyl labelled choline chloride was prepared by the method of du-Vigneaud *et al.* (5). L-C^{14}-methyl-labelled methionine was synthesized by the method of Melville, Rachele, and Keller (10). C^{14}-labelled sodium formate was prepared by a modification of the method of Melville *et al.* (10). C^{14}-labelled sodium bicarbonate was prepared by adjusting the pH of a solution of C^{14}-labelled sodium carbonate. All syntheses started from C^{14}-labelled sodium carbonate supplied by Atomic Energy of Canada, Limited.

Germination of Barley and Administration of Labelled Materials

A 720 gm. sample of Charlottetown No. 80 barley was evenly divided among twelve 20 × 25 cm. Pyrex trays. The seeds were watered twice daily and on the sixth day of germination the labelled compounds were added, dissolved in water. The barley was harvested on the 11th day of germination and worked up for the isolation of the desired compounds.

Isolation and Degradation of Hordenine

Hordenine was isolated from the roots as described previously (7). It was purified to constant specific activity by precipitation as the reineckate followed by decomposition of the salt according to Kapfhammer and Bischoff (6). This procedure effectively separates hordenine from the N-methyl tyramine which occurs along with it in Charlottetown No. 80 barley (9). The resulting hordenine hydrochloride was diluted with inactive hordenine (2.000 gm. in hydrochloric acid solution) and this aqueous solution was basified with concentrated ammonia and extracted continuously with ether for 48 hr. The ether was removed *in vacuo* and the residue converted to the methiodide as described previously (9). The methiodide was converted to the reineckate, its specific activity was checked, and the reineckate was converted to the methochloride by the procedure of Kapfhammer and Bischoff. The methochloride was then converted to the O-methyl derivative by the following procedure which gives a more complete O-methylation than that described previously (9). This results in considerably improved yields in the Hofmann degradation.

Hordenine methochloride (1.80 gm.) was dissolved in a 10% aqueous solution of sodium hydroxide (13.5 ml.) and dimethyl sulphate (9.5 ml.) was added dropwise with stirring. Acetic acid (7.9 ml.) and sodium acetate (3.9 gm.) were then added and the stirring continued for five hours and the solution left overnight. The 2-(*p*-methoxyphenyl)ethyltrimethylammonium ion was precipitated as the reineckate and its specific activity was checked. The reineckate was converted to the corresponding chloride by the method of Kapfhammer and Bischoff and this in turn was converted to the hydroxide with moist silver oxide. The quaternary hydroxide was then submitted to the Hofmann degradation as described by Leete *et al.* (9). The second product

of the degradation, *p*-vinylanisole, gave rise on oxidation to homoanisaldehyde, isolated in 26% yield as the oxime. These substances were purified to constant specific activities which are shown in Table I.

Isolation of Choline

Choline was isolated, separately, from the roots and stems of the barley as described previously (8). It was purified to constant specific activity through its reineckate salt and also through its chloroplatinate. The specific activities of these substances are reported in Table I.

Absorption of Choline Chloride by Sprouting Barley

A possible explanation of the failure of choline methyl to serve as a precursor of hordenine methyl would be that the plant does not absorb it or that it is destroyed by bacterial action before it can be utilized by the plant. In order to show that the plants were capable of absorbing choline chloride intact and translocating it throughout the plant, the following experiment was carried out.

Approximately 100 five-day-old barley seedlings were threaded through a wire screen (5 × 5 mm. mesh) in such a fashion that the stems were supported in an upright position. This prevented any possibility of the stems being externally contaminated with the labelled choline fed to the roots. The plants were fed a solution of choline chloride (2 mgm.) dissolved in water (10 ml.). The choline was labelled in the methyl group with C^{14} and the total activity fed was 5.0×10^6 counts per minute. After five days, activity could be detected in the stem tips, and radioautographs showed a distribution of activity decreasing toward the tip. The leaves were clipped off above the screen and hydrolyzed in 1 N aqueous–alcoholic potassium hydroxide solution (100 ml.) containing unlabelled carrier choline chloride (143 mgm.). Choline was isolated from the hydrolyzate as described previously (8) and purified by repeated precipitation as the reineckate (in aqueous acid) and as the chloroplatinate (in ethanol). It retained its radioactivity and held a constant specific activity (1.2×10^4 counts/min./millimole). The total activity was shown by degradation to be confined to the N-methyl groups.

This experiment demonstrates clearly that choline is absorbed by the barley root and is translocated to all parts of the plant. This translocated choline is labelled in the same fashion as the choline fed showing that there has been no randomization of activity in the absorbed molecules.

DISCUSSION

It is evident from the results in Table I that methionine methyl not only serves as a precursor of hordenine methyl but that it is more efficient in this respect than is formate. This supports the idea of Brown and Byerrum that formate serves as a precursor of methionine methyl. The previous report that choline fails to serve as a precursor of hordenine methyl is confirmed (8). This result is not an artifact caused by the exclusion of the choline by the roots or by the destruction of choline by bacteria before it could be absorbed by the roots, since choline is absorbed by the barley root and translocated to all parts of the plant. There can be no doubt, then, that there is no transfer of methyl from choline to methionine in the barley plant. It is beginning to

TABLE I

SPECIFIC ACTIVITIES

Metabolite administered	Weight, mgm.	Total activity	Specific activity in c.p.m. per mM.*						
			Hordenine	Trimethyl-amine from degradation of hordenine	Homoanisal-dehyde oxime from degradation of hordenine	Choline from root	Trimethyl-amine from degradation of root choline	Choline from stem	Trimethyl-amine from degradation of stem choline
L-Methionine	50	7.3×10^6	3.9×10^4	3.7×10^4	0	5.3×10^4	4.9×10^4	1.1×10^4	1.1×10^4
Choline chloride	50	2.5×10^7	$<0.1 \times 10^4$	Not degraded	–	–	Not degraded	Not isolated	–
Sodium formate	10	5.0×10^7	4.2×10^4	–	–	4.7×10^3	–	Not isolated	–
Sodium bicarbonate	18	5.0×10^7	$<0.1 \times 10^4$	–	–	$<0.1 \times 10^4$	–		

*All specific activities are for carrier-free material.

appear that this may be a general property of the higher plants since it has also been shown to be true in germinating *Ricinus communis* (3) and in mature *Dicentra* species (12). This gives the following picture of synthesis and transfer of labile methyl groups in the higher plants (*A* in Scheme 1) as contrasted with the process known to occur in animals (*B* in Scheme 1).
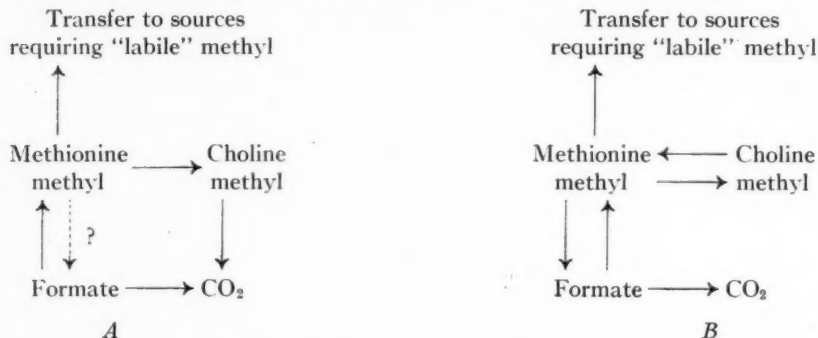
Scheme 1.   Synthesis and transfer of labile methyl in plants (*A*) and in animals (*B*).

This picture is not totally unexpected. Work with enzyme preparations from animal tissues shows that in the animal labile methyl is synthesized in the form of methionine methyl and is then transmethylated to choline (1). It has further been shown that in animals choline must first be oxidized to betaine before its methyls can be transferred back to methionine (4,11). The reason for this difference between the higher plants and the animals may be that the plant has no need to metabolize its labile methyl groups back through methionine since it has a steady supply available through its synthetic process. The animal is almost wholly dependent on its diet for its supply of labile methyl and since a good portion of this supply must be in the form of choline methyl, the possession of a mechanism for utilizing this supply has obvious "survival" value. It would appear that the animal has developed this mechanism to utilize, efficiently, the available labile methyl supply in its diet. This mechanism would appear to consist of the enzymes necessary to carry out the oxidation of choline to betaine and a "methylpherase" to carry out the transmethylation from betaine to methionine.

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THE DEPENDENCE OF THE HYDROLYSIS OF DIAZOACETIC ESTER ON THE HYDROGEN ION CONCENTRATION[1]

By A. V. Willi[2] and R. E. Robertson

ABSTRACT

The catalytic constants of the H_3O^+ catalyzed hydrolysis of diazoacetic ester in aqueous solutions containing sodium perchlorate have been determined at three different ionic strengths. A spectrophotometric method was used to follow the rate. The results indicate a strong positive salt effect. Measurements at the ionic strength $\mu = 0.110$ were carried out in the pH region from 1.97 to 5.19. For solutions containing less than 10^{-3} N perchloric acid the pH data were taken from measurements with a glass electrode in the kinetic cell. Within the limits of experimental error no deviations from proportionality between rate and $[H_3O^+]$ were found. This result is important in connection with our findings for the benzalaniline hydrolysis since it tests the methods applied and proves that the benzalaniline example with deviations from linearity between rate and $[H_3O^+]$ is a special case. The diazoacetic ester hydrolysis is not catalyzed by acetic acid molecules.

According to Brönsted's concept the rate of an acid-catalyzed reaction is determined by the sum of the catalytic effects of the different proton donators present in solution. This is formulated as:

$$k = k_0 + k_{H^+} [\text{H Solv}^+] + k_A [A]$$

where k_0 is the coefficient of the spontaneous reaction, k_{H^+} is the catalytic constant for the solvated proton (in aqueous solution the ion H_3O^+), and k_A is the constant for the catalysis of the undissociated acid.

If the reaction under consideration can only be catalyzed by hydroxonium ions or if no other catalyst except H Solv$^+$ is present, that is, if $k_A = 0$, or if $[A] = 0$, the simplified equation

$$k = k_{H^+} [\text{H Solv}^+] + k_0 \qquad [2]$$

can be applied. Furthermore, k_0 can often be neglected. While this equation has been tested for very many reactions (1), most of the experimental data for k_{H^+} have been determined in solutions of strong acids in the concentration range between 1 N and 10^{-3} N. It is generally assumed that Equation [2] is also valid for smaller H_3O^+ concentrations and consequently the same value for k_{H^+} as found in those strongly acidic solutions must be obtained at high pH values if only salt effects and medium effects can be excluded. Although this assumption may be very reasonable from the theoretical point of view, deviations from Equation [2] in unbuffered solutions have been found for the hydrolysis of benzalanilines (9) and for the decomposition of diazoacetate ion (7). In the benzalaniline example the rate was followed by a spectrophotometric method and at the same time the pH was measured with a glass electrode and a silver chloride half-cell directly introduced into the kinetic cell. The liquid junction potential of this system was considered to be negligible.

According to our knowledge, rate measurements of this type for hydrogen ion catalyzed reactions in unbuffered solutions have never been carried out before. In fact, it is generally recognized (1, p. 66) that most rate data obtained in buffer solutions of weak acids cannot be related to hydrogen ion concentrations. This is because the concentration acidity constant for the buffer at the particular ionic strength was not known and the hydrogen ion concentrations of the solutions could not be determined by potentiometric measurements since the liquid junction potentials could not be excluded. It occurred to us that deviations from linearity for the dependence of the rate on H_3O^+ in the intermediate pH range may have been observed by others, but could not be clearly established because it was not known whether their pH measurements contained systematic errors due to liquid-junction potentials. These difficulties were rendered negligible by the method applied in our study (9). Accordingly we decided to investigate a standard reaction showing acid catalysis in order to test Equation [2] for pH values higher than 3 in buffered and in unbuffered solutions. For this purpose we selected the hydrolysis of diazoacetic ester and carried out a kinetic study of the reaction $N_2CHCOOC_2H_3 + H_2O \rightarrow HOCH_2COOC_2H_5 + N_2$ for different ionic strengths in dilute aqueous solutions of perchloric acid and in buffered solutions. This reaction was studied for the first time by Fraenkel (5) and an extended investigation at different temperatures had been done by Moelwyn-Hughes and Johnson in 1941 (8). Both workers had applied the gasometric method.

EXPERIMENTAL

Diazoacetic ester was synthesized by the method given in "Gattermann" (6). Instead of the steam distillations and the subsequent vacuum distillations, we carried out only one vacuum distillation in a modified Bower-Cooke column (2).

Since diazoacetic ester has a high ultraviolet absorption band at 250 mμ ($\epsilon \approx 10,000$) (10), it was convenient to study the kinetics of the reaction by a spectrophotometric method as described in the preceding paper. The initial concentration of diazoacetic ester was 10^{-4} M or smaller and the amount of nitrogen evolved during the complete run (in a 240 cc. reaction cell) was therefore smaller than $\frac{1}{2}$ cc. and gas bubbles could not seriously influence the light absorption measurements.

The method for the pH measurements was the same as used in the preceding study for the benzalaniline hydrolysis in unbuffered solutions: a glass electrode and a silver chloride half-cell were directly introduced into the kinetic cell.

Moelwyn-Hughes and Johnson (8) have reported the formation of chloroacetic ester as a side reaction in the presence of chloride ions, consequently sodium perchlorate was used in place of potassium chloride to keep a constant ionic strength.

The Ag-AgCl half-cell contained 0.100 N sodium perchlorate and 0.0100 N potassium chloride. The ionic strength of the solutions to be measured was $\mu = 0.110$. The unbuffered solutions contained 0.110 M sodium perchlorate and the acetate buffer 0.100 M sodium perchlorate, 0.0100 M sodium acetate,

and some acetic acid. Calibration of the glass electrode was carried out in a separate temperature controlled cell containing a solution of 5.50×10^{-3} M perchloric acid and 0.1045 M sodium perchlorate with the pH of 2.26. The pH data obtained by this method refer to H_3O^+ concentrations and not to activities. The liquid-junction potentials could be neglected because the concentration differences of the various salts in both half-cells are small. Our results confirm that this assumption is justified.

RESULTS

Table I and Fig. 1 give the experimental data obtained for $k_{H^+} = k/[H_3O^+]$ in the presence of perchloric acid and sodium perchlorate at three different ionic strengths. Within each series, the k_{H^+} values agree well for different acid concentrations.

TABLE I

SALT EFFECT ON THE ACID CATALYZED HYDROLYSIS OF
DIAZOACETIC ESTER IN WATER AT 20.00°C. (± 0.03°)

No.	$C_{HClO_4} = [H_3O^+]$ (moles l.$^{-1}$)	C_{NaClO_4} (moles l.$^{-1}$)	μ (moles l.$^{-1}$)	k (min.$^{-1}$)*	k_{H^+} (min.$^{-1}$moles^{-1}l.)	Average
1	10.68×10^{-3}	0.100	0.110	0.1198	11.22	
2	5.34×10^{-3}	0.105	0.110	0.0600	11.24	11.22
3	2.862×10^{-3}	0.1075	0.110	0.03205	11.20	
4	1.431×10^{-3}	0.109	0.110	0.01607	11.23	
5	4.30×10^{-3}	0.0400	0.0443	0.0438	10.19	
6	2.862×10^{-3}	0.0411	0.0440	0.0287	(10.03)	10.20
7	1.431×10^{-3}	0.0426	0.0440	0.01459	10.20	
8	1.431×10^{-3}	0.0206	0.0220	0.01433	10.01	
9	2.150×10^{-3}	0.0200	0.0221	0.0214	9.95	9.98

*Our k values are calculated with the aid of decadic logarithms. They are defined by the formula $-(d[E]/dt) = 2.303 k[E]$.

The primary salt effect of sodium perchlorate is surprisingly large for an example of a reaction between an ion and an uncharged molecule (1, p. 23). Addition of 0.1N has been found in the present study to increase the rate at 20°C. by 11.4%, while in previous studies, at 15°C., (3) an increase of 14.8% was observed. It is noted that the special acid-catalyzed hydrolysis of dimethylacetal also exhibits a large (13%) primary salt effect of sodium perchlorate (4).

Moelwyn-Hughes and Johnson (8) investigated the diazoacetic ester reaction at different temperatures by application of the gasometric method. They found a value of $k_{H^+} = 0.3892$ [ln sec.$^{-1}$]* $= 10.14$ [log$_{10}$, min.$^{-1}$]* at 293.39°K. in 10^{-3}–10^{-2} N solutions of nitric acid without salt addition. Interpolation to 293.20°K. gives a value of $k_{H^+} = 9.94$ [log$_{10}$, min.$^{-1}$] which agrees within the experimental error with the value obtained by extrapolation of our data to zero ionic strength (9.75).

*Moelwyn-Hughes's data are defined by the equation $-(d[E]/dt) = k[E]$; our definition for k is different (see Table I).
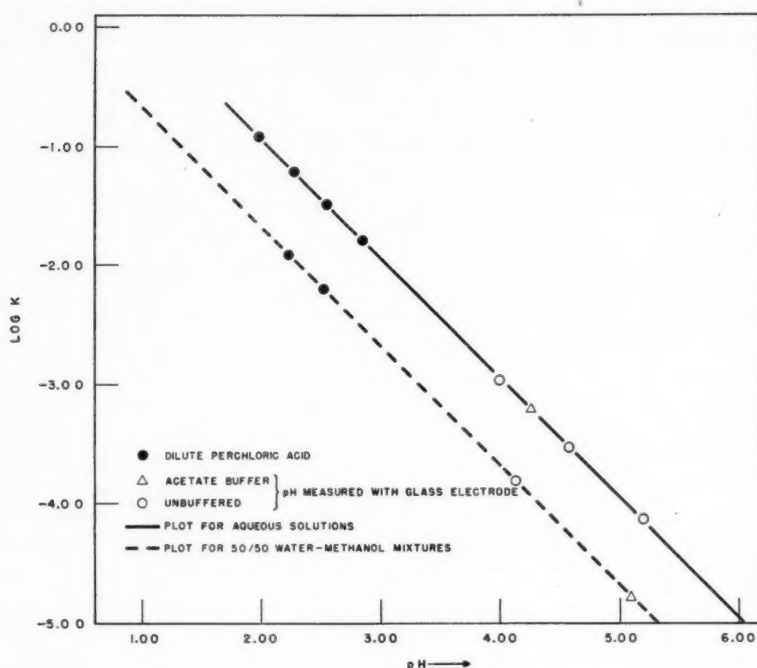
FIG. 1. The pH dependence of the rate constant for the hydrolysis of diazoacetic ester at the ionic strength $\mu = 0.110$ and 20.00°C.

TABLE II

HYDROLYSIS OF DIAZOACETIC ESTER IN ACETATE BUFFERS
AND IN UNBUFFERED AQUEOUS SOLUTIONS
$\mu = 0.110$ AT 20.00°C.

No.	Solute	pH (measured)	k (min.$^{-1}$)*	$k/[\text{H Solv}^+]$ (min.$^{-1}$moles^{-1} l.)
10	0.110 M NaClO$_4$ Trace HClO$_4$	4.00	1.08×10^{-3}	10.8
11	0.100 M NaClO$_4$ 0.0100 M NaAc 0.01947 M HAc	4.26	6.15×10^{-4}	11.2
12	0.110 M NaClO$_4$ Trace HClO$_4$	4.58	2.90×10^{-4}	11.03
13	0.110 M NaClO$_4$ Trace HClO$_4$	5.19	7.3×10^{-5}	11.3

*See Table I.

The data for the rates of decomposition of diazoacetic ester in unbuffered solutions and in acetate buffer at $\mu = 0.110$ are given in Table II and in Fig. 1. Division of the observed rate by the H_3O^+ concentration (measured with the glass electrode) gives also within the experimental error the same value for k_{H^+} as determined in the presence of 10^{-2}–$10^{-3}N$ perchloric acid at $\mu = 0.110$. For these data, an error of at least 2% may be expected because the pH could not be determined more accurately than to 0.01 units. Nevertheless, it can be concluded from these results that the equation $k = k_{H^+} [H_3O^+]$ is very well fulfilled in the pH range 2–5.2. Also in the presence of acetic acid (experiment No. 11) the observed rate is in agreement with this equation which indicates that catalysis by acetic acid is absent or negligible.

The deviations from Equation [2] for the benzalaniline reaction reported in the preceding paper were observed in a 50/50 methanol–water mixture. In order to check whether the deviations were due to the special solvent, we carried out a few experiments in the same solvent mixture on the diazoacetic ester reaction. From the data in Table III and Fig. 1 it is clear that there is also a linear relation between the rate and the concentration of the solvated protons in a 50/50 methanol–water mixture.

TABLE III

HYDROLYSIS OF DIAZOACETIC ESTER IN 50/50 w/w
METHANOL–WATER MIXTURE
$\mu = 0.110$ AT 20.00°C.

Solute	pH measured	[H Solv$^+$] (moles l.$^{-1}$)	k (min.$^{-1}$)*	k/[H Solv$^+$] (min.$^{-1}$moles^{-1} l.)
0.104 M NaClO$_4$ 5.87 × 10^{-3} M HClO$_4$		5.87 × 10^{-3}	1.23 × 10^{-2}	2.10
0.107 M NaClO$_4$ 2.93 × 10^{-3} M HClO$_4$		2.93 × 10^{-3}	6.33 × 10^{-3}	2.16
0.110 M NaClO$_4$ Trace HClO$_4$	4.13	7.4 × 10^{-5}	1.56 × 10^{-4}	2.11
0.100 M NaClO$_4$ 0.0100 M NaAc 0.01947 M HAc	5.08	8.32 × 10^{-6}	1.71 × 10^{-5}	2.06

See Table I.

These results indicate that either proportionality between rate constants and [H Solv$^+$] also exists at low hydrogen ion concentrations for a constant ionic strength, or the deviations of the rate from proportionality to [H Solv$^+$] are equal in sign and magnitude to the errors involved in our method of pH measurement. It is highly improbable that these two completely different things, the deviation of the rate from proportionality to [H Solv$^+$] and the liquid-junction potential, could be identical for a change of the independent variable by a factor of 1000. Therefore, it is justifiable to conclude: the rate is actually proportional to [H Solv$^+$] and the liquid-junction potential is in fact negligible or cancels out. The second conclusion is also important with

respect to our results obtained for the hydrolysis of benzalanilines in unbuff-
ered solutions. It indicates that our findings for the pH dependence of that
reaction are not due to deficiencies of the method of pH measurement applied
in both studies.

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REFRACTIVE INDICES OF SOME SATURATED AND MONOETHENOID FATTY ACIDS AND METHYL ESTERS[1]

By B. M. Craig[2]

ABSTRACT

The refractive indices for a number of saturated and monoethenoid fatty acids and their corresponding methyl esters have been determined. Equations have been calculated to obtain the refractive index at any given temperature. Significant differences have been found for the temperature coefficients of refractive index within an homologous series and between the free fatty acid and the corresponding methyl ester.

The literature contains few references to systematic studies on the refractive indices of the fatty acids and their esters, particularly the variation in refractive index with temperature. Dorinson, McCorkle, and Ralston (1) have reported refractive indices for the saturated fatty acids from caproic to stearic at five degree intervals of temperature. Wyman and Barkenbus (8) determined the refractive indices of the methyl esters of this series of fatty acids at 45°C. and this work was extended by Mattil and Longenecker (5) who determined the variation in refractive index as a function of temperature. The latter workers computed equations to calculate the refractive indices at any intermediate temperatures. More recently Krewson (3) has published data on the refractive indices of the methyl esters of the saturated acids up to and including methyl octacosanoate at temperatures of 50°C. and 80°C.

Data on refractive indices of unsaturated acids and esters are more meager than for the corresponding saturated series. Wood *et al.* (7) have reported on the refractive indices of oleic, elaidic, linoleic, and linolenic acids at 50°C. McCutcheon (4) determined the refractive indices of ethyl linoleate and ethyl linolenate over the range 20°C. to 60°C.

In the course of some work on rapeseed oil the saturated and monoethenoid acids and esters of the C_{16} to C_{22} series were purified, and a refractive index study was made on these materials. Equations were calculated from the data expressing the refractive index as a function of temperature.

MATERIALS AND METHODS

Crude commercial stearic acid was used as a source for methyl palmitate and methyl stearate. The crude acid was hydrogenated to a negligible iodine value and converted to methyl esters by conventional procedures. The crude esters were distilled in a Podbielniak "Heli-Grid" distillation column. The methyl palmitate and methyl stearate fractions were redistilled and fractionally crystallized to yield pure esters. Methyl oleate was obtained by converting olive oil to the methyl esters and distilling the esters. The C_{18} fraction was repeatedly crystallized from acetone until the precipitates and filtrates agreed in refractive index. The purified methyl oleate was then redistilled in

the Podbielniak column. Methyl eicosenoate and methyl erucate were prepared from rapeseed oil in the same manner. Methyl behenate and methyl arachidate were prepared from the corresponding monoethenoid esters by hydrogenation with Raney nickel followed by fractional crystallization.

The pure fatty acids were obtained by saponification of the corresponding pure methyl esters and fractional crystallization. The physical and chemical constants of the esters and acids are given in Table I. Iodine values were

TABLE I

PHYSICAL AND CHEMICAL CONSTANTS FOR FATTY ACIDS AND METHYL ESTERS

	Acids				Esters			
	I.V.	Theor. I.V.	M.P.	Reported M.P.	I.V.	Theor. I.V.	M.P.	Reported M.P.
Oleic	89.90	89.87	—	—	85.63	85.62	—	—
Eicosenoic	81.62	81.75	24.2	23–24	78.15	78.22	—	—
Erucic	74.81	74.98	33.0	33.5 (6)	71.91	71.99	—	—
Palmitic	0	0	63.3	62.9 (2)	0	0	29.9	30.6 (2)
Stearic	0	0	70.1	69.6 (2)	0	0	39.1	39.1 (2)
Arachidic	0	0	75.4	75.35 (2)	0	0	46.9	46.6 (2)
Behenic	0	0	80.7	79.95 (2)	0	0	53.3	53.3 (2)

determined by the Wijs method, one hour reaction time and 0.1 N solution. Melting points were measured by the Wiley method using a Bureau of Standards thermometer and applying stem emergence corrections. Diene and triene acid contents of the unsaturated esters and acids were determined according to the standard A.O.C.S. procedure. No measurable quantities were found in any of the monoethenoid esters or acids.

A Bausch and Lomb Abbe type refractometer equipped with a circulating water bath controlled to ± 0.005°C. was used to measure the refractive indices. A thermometer calibrated against a Bureau of Standards thermometer was used to read the temperature in the prism and stem emergent corrections were applied to the thermometer reading. No prism corrections were applied to the refractive index values. The refractive indices were measured at five degree intervals over the temperature range at which the material under study was in the liquid state from 20°C. to 85°C. The refractometer was carefully calibrated with the test piece supplied by the manufacturer and checked with purified ethyl oxalate and ethyl citrate.

RESULTS

Equations for calculation of refractive indices of the esters and acids at any given temperatures are listed in Table II. The equations were calculated by the method of least squares and the error of estimate for the equation and the standard error of the regression coefficient are included. The errors of estimate of the equations listed in Table II are of the same order as the error of measurement of refractive index credited to the instrument used in the

TABLE II

EQUATIONS FOR CALCULATION OF REFRACTIVE INDICES

		Standard error of estimate	Standard error of regression coefficient
Methyl oleate	R.I.t = 1.45968 − 0.000377t	0.00005	0.000,001
Methyl eicosenoate	R.I.t = 1.46134 − 0.000372t	0.00005	0.000,001
Methyl erucate	R.I.t = 1.46288 − 0.000369t	0.00009	0.000,002
Methyl palmitate	R.I.t = 1.44830 − 0.000379t	0.00008	0.000,002
Methyl stearate	R.I.t = 1.45149 − 0.000375t	0.00006	0.000,001
Methyl arachidate	R.I.t = 1.45363 − 0.000366t	0.00005	0.000,001
Methyl behenate	R.I.t = 1.45554 − 0.000358t	0.00007	0.000,002
Oleic acid	R.I.t = 1.46677 − 0.000354t	0.00009	0.000,002
Eicosenoic acid	R.I.t = 1.46805 − 0.000351t	0.00008	0.000,001
Erucic acid	R.I.t = 1.46892 − 0.000346t	0.00008	0.000,002
Palmitic acid	R.I.t = 1.45589 − 0.000355t	0.00006	0.000,004

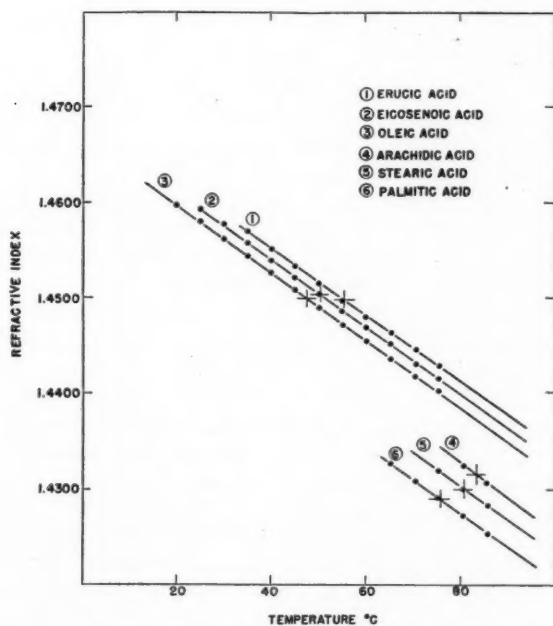*R.I. t = Refractive index at temperature tºC.*

FIG. 1.   Refractive indices of fatty acids at different temperatures.

study. Lines representing the equations and the experimental values for refractive indices are presented graphically in Figs. 1 and 2.
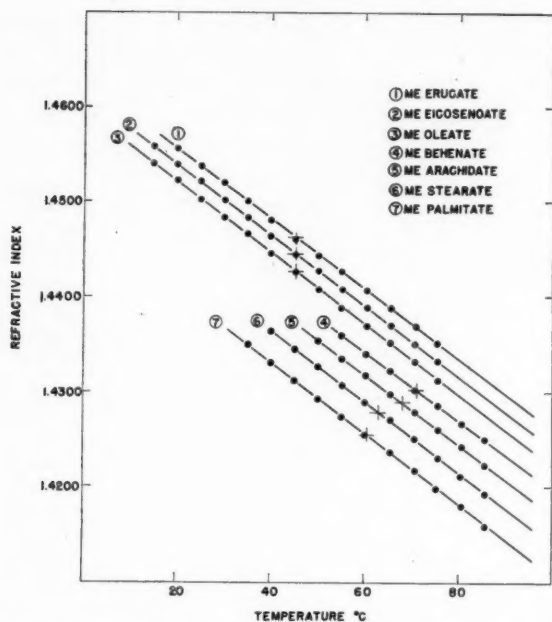
FIG. 2.   Refractive indices of methyl esters of fatty acids at different temperatures.

Table III shows a comparison of the values obtained experimentally and those calculated from the equations given in Table II. Experimental values are included for stearic, arachidic, and behenic acids where there were insufficient data to calculate the necessary equations.

The refractive indices for acids and esters given in this study agree quite well with those reported by other workers. The equations which are given in Table II represent an accurate and convenient means for calculating refractive indices at any temperature and are a definite advantage where temperatures other than those reported in the literature are used to measure refractive index. It is interesting to note that the temperature coefficients of refractive index given in the equations in Table II vary in a regular manner within an homologous series of acids or esters. Statistically the difference may not be significant between any two consecutive members of the series, e.g. methyl palmitate (0.000379) and methyl stearate (0.000375), but the difference is significant between two members such as methyl palmitate (0.000379) and methyl behenate (0.000358). It must also be noted that there are significant differences between the temperature coefficients of refractive index for the methyl esters and the corresponding free fatty acids. The practice of using the general figure 0.00038 as the temperature coefficient of refractive index will lead to an error, the magnitude of which will depend on the temperature being used.

TABLE III

COMPARISON OF CALCULATED AND EXPERIMENTAL REFRACTIVE INDICES

	Experimental	Calculated
	n_D^{25}	
Methyl oleate	1.44656	1.44650
Methyl eicosenoate	1.44833	1.44832
Methyl erucate	1.44977	1.44977
	$n_D^{60.2}$	
Methyl palmitate	1.42549	1.42550
Methyl stearate	1.42897	1.42892
Methyl arachidate	1.43165	1.43160
Methyl behenate	1.43391	1.43399
	n_D^{20}	
Oleic acid	1.45442	1.45438
Eicosenoic acid	1.45574	1.45576
Erucic acid	1.45674	1.45681
	$n_D^{55.6}$	
Palmitic acid	1.42545	1.42550

	Experimental refractive indices		
	$n_D^{75.5}$	$n_D^{80.5}$	$n_D^{55.6}$
Stearic acid	1.43202	1.43012	1.42830
Arachidic acid	—	1.43247	1.43066
Behenic acid	—	—	1.43257

The differences in the coefficient for the monoethenoid methyl ester and the corresponding saturated methyl esters, e.g. methyl oleate (0.000377), methyl stearate (0.000375), do not seem to be statistically significant. However reasoning in the same manner as for the homologous series given above, the difference might be shown to be significant if this study were extended to include diethenoid and triethenoid acids and esters of the same chain lengths.

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THE REACTION OF METHYL RADICALS WITH ISOBUTANE[1]

By M. H. Jones[2] and E. W. R. Steacie

ABSTRACT

An investigation is reported of the reaction of methyl radicals, produced in the photochemical decomposition of azomethane, with isobutane. The energy of activation of this process was found to be 6.7 ± 0.8 kcal./mole, assuming that the combination of methyl radicals has an activation energy of zero. From some experiments with n-butane, a value of 9 ± 1 kcal./mole was obtained.

INTRODUCTION

A recent study of the photolysis of azomethane (5) has shown that it may be used as a source of methyl radicals for an investigation of reactions of the type

$$CH_3 + RH \rightarrow CH_4 + R$$

where RH is any hydrogen containing compound. The choice of suitable compounds is, however, limited as the activation energy of hydrogen abstraction from azomethane itself is low. Since the method of investigation involves the determination of rate differences, the velocities of the two competitive reactions must be of roughly the same order of magnitude if quantitative results are required. Isobutane was chosen in the first instance as a simple hydrocarbon containing a tertiary carbon–hydrogen bond, where there is greater ease of abstraction (6, 7) than from a corresponding primary or secondary bond.

EXPERIMENTAL

The apparatus and analytical procedure have been described elsewhere (5). The nitrogen-methane and ethane fractions were analyzed by the mass spectrometer and there was no evidence that the latter were contaminated with isobutane.

The light source was a Hanovia S-500 medium pressure mercury arc and the collimated light beam had a volume within the quartz reaction cell of 110 cc. The cell volume was 170 cc. Two filters were used to cut out the short ultraviolet wave lengths. Filter A, a Corning filter No. 2–37 or 586, was opaque to wave lengths below 3150 Å and had a transmission such that 96% of the absorbed radiation was the 3660 Å lines. Filter B was a Corning filter No. 0–53 or 774 and limited the incident radiation to wave lengths greater than 2800 Å.

The azomethane was a sample that had been prepared previously (5). It was kept as a liquid at $-78°$ C., vapor pressure 6.6 mm., in a trap darkened to exclude daylight. Research Grade isobutane, obtained from the Phillips Petroleum Company, was found to contain traces of methane and ethane (~ 0.05 mole %) which were sufficient to cause errors of several per cent in the analysis of the reaction products. The samples used were, therefore, intro-
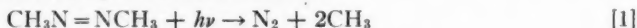
duced into the analysis system and these impurities pumped off at $-165°$ C. The n-butane, also a Research Grade product of the Phillips Company, was treated in the same manner.

RESULTS AND DISCUSSION

The rates of formation of the gaseous products in the photolysis of azomethane may be described by the reaction scheme

$$CH_3N = NCH_3 + h\nu \rightarrow N_2 + 2CH_3 \qquad [1]$$

$$CH_3 + CH_3N = NCH_3 \rightarrow CH_4 + CH_2N = NCH_3 \qquad [2]$$

$$CH_3 + CH_3 \rightarrow C_2H_6 . \qquad [3]$$

In the presence of isobutane we have the additional methane producing reaction

$$CH_3 + C_4H_{10} \rightarrow CH_4 + C_4H_9 . \qquad [4]$$

From reactions [2] and [3] it follows that, when R_{CH_4} and $R_{C_2H_6}$ are the rates of formation of methane and ethane, for azomethane alone

$$R_{CH_4} = k_2 [CH_3] [Azo]$$

$$R_{C_2H_6} = k_3 [CH_3]^2.$$

The other terms in the rate equations have their usual significance. Hence, applying the normal stationary state conditions,

$$(R_{CH_4}/R_{C_2H_6}^{1/2})_A = k_2 [Azo]/k_3^{1/2}$$

and for the photolysis of a mixture of azomethane and isobutane,

$$(R_{CH_4}/R_{C_2H_6}^{1/2})_{A+B} = k_2 [Azo]/k_3^{1/2} + k_4 [C_4H_{10}]/k_3^{1/2}$$

where the subscripts A and B refer to the reactants. As the quantities $(R_{CH_4}/R_{C_2H_6}^{1/2})_{A+B}$ and $(R_{CH_4}/R_{C_2H_6}^{1/2})_A$ may be determined over a range of temperatures for known concentrations of azomethane and isobutane on the one hand, and azomethane on the other, we have a method of obtaining values of $(R_{CH_4}/R_{C_2H_6}^{1/2})_B/[C_4H_{10}]$ i.e. $k_4/k_3^{1/2}$. Thus expressing the rate coefficients in terms of the Arrhenius parameters,

$$k_4/k_3^{1/2} = (A_4/A_3^{1/2}) \exp[-(E_4 - \tfrac{1}{2}E_3)/RT] .$$

Such a kinetic treatment is dependent on the condition that methane is formed only by reactions [2] and [4] and ethane only by reaction [3].

The values of $(R_{CH_4}/R_{C_2H_6}^{1/2})_B/[C_4H_{10}]$ at temperatures between $24°$ C. and $169°$ C. are given in Table I. The values of $(R_{CH_4}/R_{C_2H_6}^{1/2})_A/[Azo]$ required in these calculations have been taken from the experimental Arrhenius curve for azomethane which was found to deviate from linearity at temperatures below $80°$ C. The choice of the experimental rather than the extrapolated curve in this region is considered justified by the fact that the resultant Arrhenius plot for isobutane is linear over the whole temperature range. The set of experiments, runs 111–127, on which the azomethane plot is based were conducted immediately following those quoted in Table I with Filter A and immediately prior to those with Filter B, using the same sample of azomethane. The optical system was undisturbed.

TABLE I

ABSTRACTION OF HYDROGEN ATOMS BY METHYL RADICALS FROM ISOBUTANE AND n-BUTANE

Run No.	Temp., °K.	$[Azo].10^6$ mole/cc.	$[C_4H_{10}].10^6$ mole/cc.	$R_{S_2}.10^5$	$R_{CH_4}.10^5$	$R_{C_2H_6}.10^5$	$\dfrac{(R_{CH_4}/R_{C_2H_6}^{1/2})_{A+B}.10^3}$	$\dfrac{(R_{CH_4}/R_{C_2H_6}^{1/2})_B.10^{-2}}{[C_4H_{10}]}$
				cc. at N.T.P./min.				
Filter A								
Isobutane								
104	298	3.22	3.38	50.2	6.91	35.8	3.66	2.87
107	300	2.25	4.59	35.0	6.02	26.2	3.72	3.75
99	319	2.13	2.37	39.0	6.50	22.1	4.36	5.87
103	334	2.81	3.16	42.4	12.8	22.6	8.53	9.37
100	345	2.55	2.64	44.6	12.6	14.9	10.3	14.2
109	356	2.37	2.58	42.8	14.3	11.9	13.1	19.6
101	373	2.73	2.57	48.9	19.1	7.40	22.2	26.5
110	384	2.63	2.67	44.5	21.4	4.51	31.8	43.8
106	404	2.13	2.89	41.3	22.2	2.85	41.5	54.3
102	406	2.51	2.34	46.2	22.6	2.36	46.5	58.5
105	427	2.17	2.13	42.8	24.7	1.45	64.8	93.0
108	442	2.12	2.20	40.4	26.7	1.09	81.2	98.6
Filter B								
Isobutane								
128	328	2.85	3.03	222.9	31.9	155.1	8.10	10.6
130	349	2.53	2.59	215.9	43.5	123.3	12.4	20.0
142	354	2.71	2.65	242.8	44.0	118.5	12.8	15.4
137	375	2.71	2.36	218.0	67.1	83.4	23.2	30.9
125	384	2.33	2.47	218.5	78.4	55.8	33.2	61.9
132	411	2.50	2.51	228.8	102.4	26.2	63.3	106.
135	438	2.38	2.35	221.0	123.4	17.5	93.5	134.
n-Butane								
141	352	2.37	2.77	210.5	32.7	112.5	9.76	9.28
136	375	2.45	3.03	204.4	57.3	75.6	20.8	21.1
133	411	2.42	2.61	221.9	87.8	26.8	53.5	69.0
134	435	2.73	2.83	254.	122.5	15.8	97.2	111.

As the rates have been given in cc. of gas at N.T.P./min. and the concentrations in mole/cc., the $(R_{CH_4}/R_{C_2H_6}^{\frac{1}{2}})_B/[C_4H_{10}]$ values must be multiplied by the factor $1.11.10^{-15}/V^{\frac{1}{2}}$ where V is the reaction volume, in order to express $k_4/k_3^{\frac{1}{2}}$ in the more conventional units (molecule/cc.)$^{-\frac{1}{2}}$ sec.$^{-\frac{1}{2}}$
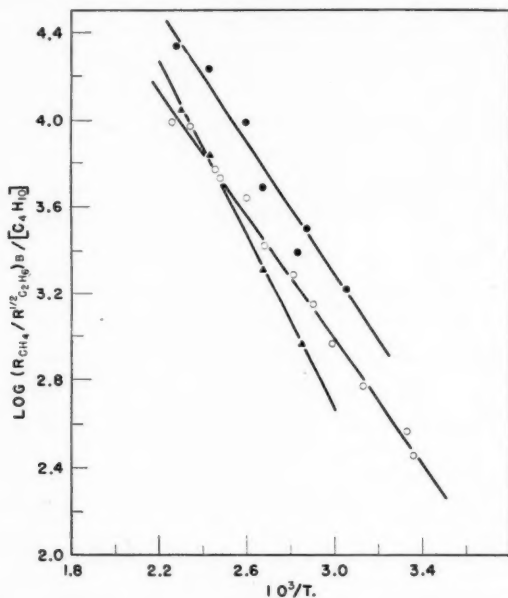

FIG. 1.   Arrhenius plots for the reaction of methyl radicals with the butanes. The results for isobutane with Filter B have been displaced upwards 0.2 units in the ordinate scale.
Filled Circles—isobutane—Filter B.
Open Circles—isobutane—Filter A.
Triangles—n-butane.

The Arrhenius curves for isobutane appear in Fig. 1 together with that obtained from four experiments with n-butane. The activation energy differences, $E_4 - \frac{1}{2}E_3$, calculated by the method of least squares are recorded in Table II. These are the values for the hydrogen abstraction reaction if it is assumed that the combination of methyl radicals does not require activation (3). The difference between the two results for isobutane is not considered significant. Thus, taking the mean, a value of 6.7 ± 0.8 kcal./mole is obtained, and for the reaction of methyl with n-butane, 9 ± 1 kcal./mole. Although the standard errors quoted above are greater than those determined from the experimental scatter (see Table II), we consider that they represent a more conservative estimate of the errors inherent in the system. The ratio of the steric factors, $P_4/P_3^{\frac{1}{2}}$, calculated from simple collision theory ($A = PZ$ in the Arrhenius expression) are 10^{-4} and 10^{-3} respectively for isobutane and n-butane, assuming the collision diameters $CH_3 = 3.5.10^{-8}$ cm., iso-C_4H_{10} $= 5.8.10^{-8}$ cm., and n-$C_4H_{10} = 5.9.10^{-8}$ cm.

TABLE II

COMPARISON OF RESULTS OBTAINED IN VARIOUS INVESTIGATIONS FOR THE
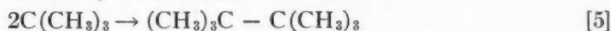REACTION OF METHYL RADICALS WITH ISOBUTANE AND n-BUTANE

Compound	Methyl source	$E_4 - \frac{1}{2}E_3$, kcal.	$P_4/P_3^{\frac{1}{2}}$	$k_4/k_3^{\frac{1}{2}}.10^{13}$ at 100°C., molecule$^{-\frac{1}{2}}$.cc$^{\frac{1}{2}}$.sec.$^{-\frac{1}{2}}$	Reference
Isobutane	Azomethane. Filter A	6.5 ± 0.2	$\sim 10^{-4}$	3.0^a	This work
Isobutane	Azomethane. Filter B	6.9 ± 0.5	$\sim 10^{-4}$	4.0^a	This work
Isobutane	Acetone	7.6	3.10^{-4}	6.2	Trotman-Dickenson, Birchard, and Steacie (7)
Isobutane	Mercury dimethyl	(4.2)			Smith and Taylor (6)
n-Butane	Azomethane. Filter B	9.1 ± 0.3	$\sim 10^{-3}$	2.2^a	This work
n-Butane	Acetone	8.3	3.10^{-4}	2.7	Trotman-Dickenson, Birchard, and Steacie (7)
n-Butane	Mercury dimethyl	(5.5)			Smith and Taylor (6)
n-Butane	Mercury dimethyl	8.4			Gomer (2)

^{a} *The values of $k_4/k_3^{\frac{1}{2}}$ have been calculated on the assumption that the effective reaction volume is that of the light beam i.e. 110 cc.*

Also included in Table II are the activation energies for the abstraction of a hydrogen atom from the two butanes by methyls produced in the photolysis of acetone (7) and mercury dimethyl (6, 2). Except for the earlier values of Smith and Taylor, which are inaccurate since they were based on the rate of methane formation only, assuming the ethane rate remained sensibly constant, there is fair agreement in the results. It may be concluded, therefore, as is to be expected, that the reaction is independent of the nature of the methyl source. Since the method of kinetic analysis is identical when azomethane and acetone are used as the radical source, it is possible to make a comparison of the ratio of the rate coefficients, $k_4/k_3^{\frac{1}{2}}$, which does not reflect compensating error in E and A. Here there is also good agreement, particularly in the case of n-butane.

Trotman-Dickenson and Steacie (8) have demonstrated that the facility with which a hydrogen atom may be abstracted from a hydrocarbon increases in the order primary, secondary, and tertiary. In a compound containing more than one type it is at once apparent that the measured E is a composite activation energy. However, even for isobutane, which possesses only one tertiary and nine primary hydrogen atoms, the abstraction is predominately tertiary and at temperatures below 200° C. a simple calculation shows that the deviation in the energy of activation lies within the experimental accuracy. The same is true of n-butane where the abstraction is mainly secondary.

The radicals resulting from reaction [4] are the tertiary butyl and secondary butyl. As these are stable thermally up to 250° C. (1, 4) it can be supposed that they disappear either by combination with each other

$$2C(CH_3)_3 \rightarrow (CH_3)_3C - C(CH_3)_3 \qquad [5]$$

and $\qquad 2CH_3CH_2CHCH_3 \rightarrow CH_3CH_2(CH_3)CH - CH(CH_3)CH_2CH_3 \qquad [6]$

or with methyl radicals

$$(CH_3)_3C + CH_3 \rightarrow (CH_3)_3C \cdot CH_3 \qquad [7]$$

and $CH_3CH_2CHCH_3 + CH_3 \rightarrow CH_3CH_2CH(CH_3)_2$.   [8]

Evidence for a reaction analogous to [7] and [8] accounting for the fate of the radical $CH_2N = NCH_3$ was obtained in the study of the photolysis of azomethane alone (5). A disproportionation reaction between butyl radicals may also occur although indirect evidence suggests that the corresponding unsymmetrical process involving a methyl does not take place (7). As the latter is a methane producing reaction, the kinetic method described above would be invalidated if it occurred to any appreciable extent.

ACKNOWLEDGMENT

The authors are indebted to Dr. F. P. Lossing and Miss F. Gauthier for the mass spectrometer analyses.

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UNIT CELL, SPACE GROUP, AND X-RAY POWDER DIFFRACTION DATA FOR LYCOCTONINE MONOHYDRATE, $C_{25}H_{39-41}O_7N.H_2O$[1]

BY W. H. BARNES AND MARIA PRZYBYLSKA*

Recently we have had occasion to take X-ray powder photographs of crystals of lycoctonine monohydrate, $C_{25}H_{39-41}O_7N.H_2O$ (2), provided by Dr. L. Marion, and extracted from *Delphinium consolida* L. (3) and from *Delphinium Brownii* Rydb. (4), respectively. The diffraction patterns were identical but it was difficult to compare our photographs directly with the prints reproduced by Cook and Beath (1) for a specimen isolated from *Delphinium barbeyi* H., and for one prepared by the basic hydrolysis of ajacine. Dr. W. B. Cook, however, very kindly supplied a sample of his lycoctonine monohydrate from *D. barbeyi*, and a powder photograph confirmed its identity with the specimens from *D. consolida* and from *D. Brownii*.

In order to obtain additional X-ray crystallographic data for lycoctonine monohydrate, the unit cell constants and space group have been determined from precession photographs with filtered Cu radiation ($\lambda(K_\alpha) = 1.5418$Å).

Lycoctonine monohydrate is monoclinic sphenoidal and crystallizes from aqueous alcohol as colorless needles, elongated in the direction of the b axis. Radial aggregates are common. The most prominent forms observed were {110}, {10$\bar{1}$}, and {100}. The crystals are strongly piezoelectric. The unit cell dimensions are $a = 14.95 \pm 0.03$Å, $b = 7.92 \pm 0.02$Å, $c = 10.98 \pm 0.02$Å, $\beta = 103°6'$. The space group is $P2_1$ (C_2^2) and there are two molecules per cell. The molecule of lycoctonine monohydrate, therefore, is asymmetric.

The density, as found by flotation in aqueous potassium iodide solutions, was 1.26 gm. per ml. at room temperature. That calculated for $C_{25}H_{39}O_7N.H_2O$ is 1.267 gm. per ml. while that for $C_{25}H_{41}O_7N.H_2O$ is 1.273 gm. per ml., thus precluding a choice between H_{39} and H_{41} on this basis unless relatively large clear crystals, suitable for very accurate density measurements, can be grown. The observed density, however, supports evidence (4) that the crystals are the monohydrate since the calculated density of the dihydrate (H_{39} or H_{41}) is approximately 1.31 gm. per ml.

X-ray powder diffraction data are given in Table I. Lines corresponding to $d > 3.20$Å have been indexed on the basis of the unit cell constants. The powder patterns were obtained with Co radiation ($\lambda(K_\alpha) = 1.790$Å), filtered with Fe foil, in a 114.6 mm. diameter camera using Straumanis film mounting. The longest interplanar spacing measurable with the apparatus used (i.e., the "cutoff") was 20Å. Film shrinkage corrections were only about 0.13% and, therefore, were not applied. Relative intensities (I/I_1) were estimated visually.

TABLE I

X-Ray Powder Diffraction Data for Lycoctonine Monohydrate

I/I₁	d(Å) Obs.	d(Å) Calc.	hkl	I/I₁	d(Å) Obs.	d(Å) Calc.	hkl
80	14.5	14.56	100				
10	10.6	10.70	001			3.57	003
80	9.69	9.74	$10\bar{1}$	15	3.48	3.53	$121, 20\bar{3}$
85	7.79	7.81	101			3.50	212
50	7.29	7.28	200			3.48	220
100	6.94	6.96	110	30	3.40	3.42	$22\bar{1}$
—	—	6.77	$20\bar{1}$			3.39	$40\bar{2}$
50	6.36	6.37	011	5	3.31	3.32	$11\bar{3}$
40	6.10	6.14	$11\bar{1}$			3.31	410
—	—	5.56	111	15	3.20		
		5.47	201	2	3.06		
30	5.37	5.43	$10\bar{2}$	5	3.00		
		5.36	210	20	2.85		
		5.35	002	20	2.68		
25	5.14	5.15	$21\bar{1}$	1	2.59		
12	4.85	4.87	$20\bar{2}$	5	2.54		
		4.85	$300, 30\bar{1}$	5	2.49		
3	4.68	4.69	$10\bar{2}$	4	2.43		
		4.50	211	2	2.37		
30	4.44	4.48	$11\bar{2}$	4	2.32		
		4.43	012	3	2.25		
		4.15	$21\bar{2}$	3	2.22		
50	4.13	4.14	$310, 31\bar{1}$	2	2.17		
		4.08	$301, 30\bar{2}$	4	2.15		
12	4.03	3.96	020	2	2.07		
1	3.92	3.91	202	1	2.05		
20	3.81	3.82	120	2	1.94		
—	—	3.71	$021, 40\bar{1}$	1	1.88		
		3.67	$12\bar{1}$	5	1.83		
10	3.65	3.66	$10\bar{3}$	1	1.78		
		3.64	400				
		3.63	311				

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THE OXIDATION OF FERROUS SULPHATE SOLUTIONS BY γ-RAYS— THE EFFECT OF SURFACE/VOLUME RATIO OF POLYSTYRENE CELLS ON THE YIELD[1]

By T. J. Hardwick

A serious discrepancy exists between the results of various workers on the yield of ferric ion in the oxidation of air-saturated ferrous sulphate solutions in 0.8 N sulphuric acid by γ-rays. Hochanadel (5) found a value of G (mols. ferric ion produced per 100 ev. absorbed) = 15.5, using a calorimetric method to determine the rate of energy absorption. Using air wall equivalent ion chambers for the measurement of energy absorbed, Miller (6) found G = 20.4, while Hardwick (3) obtained G = 20.8.

A possible reason for this anomaly was that while Hochanadel carried out his irradiations in glass vessels, Miller and Hardwick used plastic (polystyrene)

cells. It has been shown that traces of organic material seriously interfere with the oxidation of ferrous ion by radiation, generally causing an increase in yield (1,2,4). It was therefore a possibility that the bombardment of the plastic interface by the electrons produced within the wall might pollute the solution sufficiently to cause a change in the measured yield.

The existence of such an effect could be checked if irradiations were made in plastic cells with varying surface/volume ratios. The amount of impurity going into solution would be dependent on the surface area only. Under conditions of constant surface area and varying volume, any effect arising from the plastic walls would be greatest in the smallest volume.

Three sets of well-type cells were constructed of polystyrene, an identical pair to each set. The general features are shown in Fig. 1; a more detailed
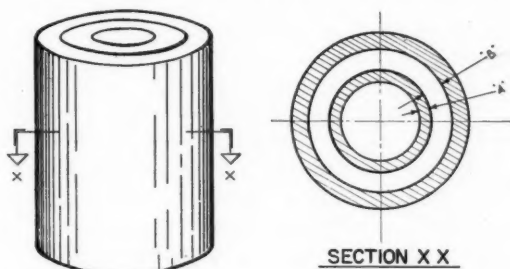

SECTION X X

FIG. 1. Well-type cell used for irradiations.

description has been reported previously (3). The dimension A refers to the thickness of plastic between the source and the irradiation chamber, while B refers to the width of the annulus comprising the irradiation volume. All other dimensions were the same in all cells.

TABLE I

Cell No.	Dimension A, mm.	Dimension B, mm.	Surface, cm.^{2}	Volume, cm.^{3}	Surface/Volume, cm.$^{-1}$	Yield, mols./100 ev.
1	2.37	3.89	61.03	10.80	5.65	20.8 ± 0.3
2	3.85	2.41	62.14	7.07	8.79	20.5 ± 0.5
3	4.66	1.60	62.64	4.83	10.97	20.2 ± 0.8

The method of using one of each pair of cells for ion chamber measurements and the other for solution irradiations has been described previously (3). The results are shown in Table I. The values of the yield are based on at least 10 ferrous ion oxidations each and as many ion current measurements. The standard deviation becomes greater at smaller volumes owing to the technical difficulty in working with such small annuli. Clearly there is no marked effect of the surface volume ratio on the yield. Pollution of the solution by the walls, if it occurs, does not appear to affect the yield. Another explanation must be sought for the discrepancies between the two methods of determining the yield in this chemical system.

¹ *Issued as A.E.C.L. No. 41.*

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UNIT CELL, SPACE GROUP, AND X-RAY POWDER DIFFRACTION DATA FOR THE ALKALOID LUPANOLINE, $C_{15}H_{24}N_2O_2$, AND FOR LUPANOLINE MONOHYDRATE, $C_{15}H_{24}N_2O_2.H_2O$[1]

BY W. H. BARNES AND MARIA PRZYBYLSKA*

In connection with X-ray crystallographic studies of the C_{15} lupin alkaloids (3), lupanoline, $C_{15}H_{24}N_2O_2$, was examined at a time when the possibility of its identity with hydroxylupanine was under consideration. Recently (2) the former has been fully characterized chemically as an isomer of the latter. During an exploratory X-ray investigation, it became apparent that crystals from two authentic specimens of lupanoline were not structurally identical. They have now been identified as anhydrous lupanoline and lupanoline monohydrate. Unit cell, space group, and X-ray powder diffraction data are presented as an aid to their differentiation.

Work was commenced with crystals of a specimen that may be designated lupanoline A, and a few preliminary X-ray precession photographs were obtained. Owing to the pressure of other matters, however, some months elapsed before the study of lupanoline was continued with another, presumably identical, group of crystals. Precession photographs showed, however, that the crystals of this second specimen (lupanoline B) were not structurally the same as those of lupanoline A. The problem temporarily became more confused by the discovery that three or four selected single crystals of lupanoline B that had been stored in a separate vial had become opaque and gave, without crushing, excellent *powder* diffraction patterns. X-ray photographs taken before and after exposure of lupanoline B crystals to heat, sunlight, and mechanical grinding showed that no polymorphic change could be induced by these agencies. In the meantime, attempts to determine the density of lupanoline B by flotation of the clear crystals in aqueous potassium iodide solutions had been troublesome, and it was observed that these crystals also had become opaque. Immersion of clear single crystals of lupanoline B in distilled water, and simple exposure to water vapor in a closed vial, provided the answer. Single crystals of lupanoline B take up water of hydration very readily to form hydrate pseudomorphs consisting of sufficiently large numbers of submicroscopic crystals in completely random orientation to give almost ideal X-ray diffraction powder patterns even without rotation of the specimen. Apart from opacity, the sharply defined faces of the original crystals are retained and the pseudomorphs exhibit no marked evidence of friability.

The identity of the hydrate of lupanoline B with lupanoline A was estab-

lished by a series of powder photographs of both substances after wetting, and after drying at about 60°C., care being taken to prevent loss, or gain, of water until each photograph had been obtained.

That lupanoline B is anhydrous, and is not merely a lower hydrate, is evident from the analytical results for the sublimed alkaloid (1). The degree of hydration of lupanoline A was difficult to determine because, although water can be removed at a relatively low temperature, the crystals vaporize readily. Through the courtesy of Dr. L. Marion a determination of C and H was obtained on a sample (3.856 mgm.) of lupanoline (A) that had been exposed for 48 hr. to an atmosphere saturated with water vapor. The results are shown in Table I where they may be compared with those found for

TABLE I

LUPANOLINE

	Found (%)		Calculated (%)		
	A	B	Dihyd.	Monohyd.	Anhyd.
C	62.6	69.0	60.0	63.8	68.1
H	8.94	8.85	9.33	9.28	9.15

sublimed lupanoline (B) and those calculated for anhydrous $C_{15}H_{24}O_2N_2$, for the monohydrate, and for the dihydrate.

The analytical results for lupanoline A are low probably owing to the presence of excess water which was difficult to remove completely without danger of partial dehydration of the hydrate. There is no doubt, however, that lupanoline A is lupanoline monohydrate and that lupanoline B is anhydrous.

Anhydrous lupanoline (B) and lupanoline monohydrate (A) both crystallize from acetone as clear, colorless, monoclinic prisms elongated in the direction of the b axis, and as flat rectangular plates tabular on $(10\bar{1})$. Both are strongly piezoelectric. Their densities were difficult to measure owing to the small size of the crystals, but that of anhydrous lupanoline was estimated to be less than 1.23 gm. per ml. by flotation in solutions of ethylene dichloride and amyl acetate, and that of lupanoline monohydrate as approximately 1.25 gm. per ml. by flotation in solutions of amyl bromide and methyl iodide.

Unit cell and space group data, obtained from precession photographs, are given in Table II. Both Mo (Zr filtered), λ (K_α) = 0.7107 Å, and Cu (Ni

TABLE II

UNIT CELL AND SPACE GROUP DATA FOR LUPANOLINE AND FOR LUPANOLINE MONOHYDRATE

	Lupanoline (anhydrous)	Lupanoline monohydrate
a	11.97 ± 0.02 Å	10.59 ± 0.02 Å
b	12.20 ± 0.02 Å	8.06 ± 0.02 Å
c	10.73 ± 0.02 Å	9.03 ± 0.02 Å
β	114°9′	105°14′
S.G.	$P2_1$ (C_2^2)	$P2_1$ (C_2^2)
Mols./cell	4	2
Density (calc.)	1.226 gm./ml.	1.260 gm./ml.

TABLE III

X-RAY POWDER DIFFRACTION DATA FOR LUPANOLINE AND FOR LUPANOLINE MONOHYDRATE

Lupanoline (anhydrous)				Lupanoline monohydrate			
I/I_1	d(Å) Obs.	d(Å) Calc.	hkl	I/I_1	d(Å) Obs.	d(Å) Calc.	hkl
4	10.8	10.92	100	20	10.2	10.23	100
—	—	9.78	001	30	8.73	8.70	001
90	9.45	9.46	$10\bar{1}$	30	7.70	7.70	$10\bar{1}$
15	8.11	8.14	110	100	6.30	6.33	110
20	7.62	7.63	$01\bar{1}$	40	5.90	5.91	011, 101
—	—	7.47	$1\bar{1}1$	50	5.53	5.57	$1\bar{1}1$
25	6.13	6.14 / 6.10	101 / $02\bar{0}$	85	5.09	5.12 / 5.02	200 / $20\bar{1}$
—	—	5.91	$20\bar{1}$	40	4.76	4.76	$11\bar{1}$
10	5.50	5.49 / 5.46	111 / 200	10	4.45	4.45	$10\bar{2}$
20	5.35	5.36	$10\bar{2}$	—	—	4.35	002
—	—	5.32	$21\bar{1}$, 120	25	4.30	4.32 / 4.26	210 / $21\bar{1}$
100	5.15	5.18 / 5.13	021 / $12\bar{1}$	10	4.00	4.03 / 3.98	020 / $20\bar{1}$
35	4.90	4.99 / 4.91 / 4.89	210 / $11\bar{2}$ / $00\bar{2}$	15	3.89	3.90	$11\bar{2}$
1	4.72	4.73	$20\bar{2}$	5	3.82	3.85 / 3.83	$20\bar{2}$ / 012
15	4.55	4.54	012	2	3.72		
—	—	4.41	$21\bar{2}$	20	3.66		
5	4.32	4.33	121	18	3.56		
10	4.23	4.24	$22\bar{1}$	5	3.48		
25	4.08	4.11 / 4.07 / 4.03	201 / 220 / $12\bar{2}$	40	3.32		
—	—	3.99	$30\bar{1}$	30	3.15		
7	3.90	3.91 / 3.89	102 / 211	5	2.97		
30	3.73			10	2.77		
12	3.56			2	2.70		
5	3.41			15	2.64		
5	3.34			1	2.56		
12	3.26			10	2.51		
3	3.12			5	2.44		
3	3.07			10	2.36		
1	2.97			5	2.29		
15	2.90			25	2.25		
10	2.82			2	2.22		
1	2.73			2	2.20		
10	2.65			5	2.16		
1	2.58			2	2.10		
20	2.50			2	2.04		
7	2.36			3	1.95		
8	2.32			1	1.91		
2	2.26			8	1.87		
7	2.20			3	1.78		
1	2.12			1	1.75		
3	2.08			1	1.71		
1	2.04			2	1.66		
7	2.00			2	1.51		
2	1.94						
2	1.87						
1	1.77						
1	1.72						

filtered), λ $(K_\alpha) = 1.5418$ Å, radiations were employed and measurements were corrected for film shrinkage. An interesting feature of the unit cell data is the presence of four molecules per cell in anhydrous lupanoline. Since the maximum number of asymmetric units per cell in $P2_1$ is 2, two molecules of lupanoline must occupy one set of general positions and two molecules must occupy another set of general positions. The asymmetric unit, therefore, may be considered as comprising two molecules of lupanoline.

X-ray powder diffraction data are shown in Table III. The photographs were taken with Co radiation (λ $(K_\alpha) = 1.790$ Å), filtered with Fe foil, in a camera of 114.6 mm. diameter, and with Straumanis film mounting. The longest interplanar spacing measurable with the apparatus used (i.e., the 'cut-off') was 20 Å. Film shrinkage corrections were $< 0.2\%$ and were not applied. Relative intensities (I/I_1) were estimated visually. Lines having values of $d > 3.8$ Å have been indexed on the basis of the single crystal data given in Table II.

We are indebted to Mrs. H. M. Sheppard for assistance with the powder investigations.

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SYNTHESIS OF C^{14} LABELED DDT

BY R. MACDONALD AND B. E. BAKER

Barry (1) described a method for preparing DDT which involved the coupling of acetic anhydride with chlorobenzene by the use of aluminum chloride catalyst to yield p-chloroacetophenone. The 1-(p-chlorophenyl)-2,2,2-trichloro-ethanone, which was obtained by direct chlorination of p-chloroacetophenone, yielded the corresponding alcohol on reduction with aluminum isopropoxide in dry isopropyl alcohol. The alcohol was condensed with chlorobenzene to give DDT.

This note describes the adaptation of this method to the preparation of C^{14} labeled DDT.

EXPERIMENTAL

The complete synthesis was carried out with inactive materials and the biological toxicity of the product was compared with an authentic sample of the insecticide. The biological tests showed that the probability of the two materials being different was approximately 1 in 100. It may be concluded that the materials were practically identical so far as their biological effects in these tests were concerned. A mixed melting point of the two materials remained unchanged.

Preparation of Sodium Acetate-1-C[14]

Labeled barium carbonate was prepared by the technique described by Henneberry and Baker (2). Forty milliliters of sodium carbonate (C[14]) solution, having an activity of 0.211 mc. per ml., along with 203 mgm. of inactive sodium carbonate, was added to 50 ml. of saturated barium hydroxide solution. The water and alcohol used to wash the barium carbonate were combined and set aside for measurement of radioactivity (A). The yield of barium carbonate (C[14]) was 1.976 gm.

Labeled sodium acetate was prepared from the barium carbonate following the procedure of Van Bruggen et al. (5). The barium carbonate (1.976 gm.) was transferred to the reaction vessel by means of acetone (B) which was subsequently evaporated. At the end of the reaction the ether and residual carbon dioxide were removed by a stream of nitrogen gas. The flushing gas was bubbled through $1N$ sodium hydroxide (C). The residue (D), left in the reaction vessel after the acetic acid had been removed by distillation, was also kept for measurement of radioactivity. The yield of anhydrous sodium acetate was 0.6080 gm.

Preparation of Acetic Anhydride-1-C[14] and p-Chloroacetophenone-carbonyl-C[14]

A mixture of sodium acetate-1-C[14] (0.6080 gm.) and p-toluene sulphonyl chloride (1.62 gm.) was heated at 180–200°C. for 30 min. This method of preparing acetic anhydride from sodium acetate was described briefly by Shantz and Rittenberg (4). The acetic anhydride which distilled off (residue E) was heated at 100°C. for 18 hr. with anhydrous aluminum chloride (2.4 gm.) and chlorobenzene (1.40 gm.). At the end of this heating period, ice (4 gm.) and concentrated sulphuric acid (4 ml.) were added and the temperature was reduced to 50–60°C. The p-chloroacetophenone-carbonyl-C[14] was recovered from the reaction mixture by steam distillation (residue F). It was extracted from the aqueous layer (G) with ether and was distilled under reduced pressure. The ether forerun and the residue were combined and kept for measurement of radioactivity (H).

Preparation of 1-(p-Chlorophenyl)-2,2,2-trichloroethanol-1-C[14]

The 1-(p-chlorophenyl)-2,2,2-trichloroethanone-1-C[14], which was obtained by the chlorination at 210°C. of labeled p-chloroacetophenone, was mixed with anhydrous isopropanol (8 ml.) and aluminum isopropoxide (2.16 gm.). The temperature of the reaction mixture was maintained sufficiently high to remove the acetone as it was formed. When a negative test (2,4-dinitrophenylhydrazine reagent) for acetone in the distillate was noted, the reaction mixture was refluxed for 15 min. and the distillation was then continued. The cycle of distillation, refluxing, and distillation, was repeated until the test for acetone in the distillate (I) was no longer positive. Ice (3 gm.), followed by concentrated sulphuric acid (3 ml.), was added to the reaction mixture and the product was extracted with ether and then was distilled under reduced pressure. The residue (J) was kept for measurement of radioactivity.

Preparation of 1,1-bis(p-chlorophenyl)-2,2,2-trichloroethane-1-C[14]

Chlorobenzene (0.6 gm.) and sulphuric acid (3.84 ml., 99.1% pure) were added to the flask which contained the labeled 1-(p-chlorophenyl)-2,2,2-

trichloroethanol. The reaction vessel was surrounded by an ice bath and the reactants were stirred for six hours. The reaction was quenched by the addition of ice (1 gm.) and the DDT was extracted with four 5-ml. portions of chloroform. The acid layer (K) was kept for measurement of radioactivity. The DDT solution was evaporated (distillate L) to dryness at 60° at 1 mm. The crude DDT was purified by three successive recrystallizations from ethanol. There was obtained 197 mgm. of DDT melting at 108°–108.7° (corr.) and having a specific activity of 62,000 counts per min. per mgm. The mother liquors (M), from the recrystallization, were combined and set aside for measurement of radioactivity.

The oxidation mixture of Lindenbaum *et al.* (3) was found to be inadequate for the complete combustion of DDT. Reproducible results were obtained when 20 ml. of a solution containing 69 gm. of chromic trioxide, 200 ml. of fuming sulphuric acid (30% SO_3), and 100 ml. of syrupy phosphoric acid was used for each sample.

The residues, distillates, washings, etc., which were obtained during the synthesis, were dissolved in appropriate solvents, and aliquots were oxidized with the modified Lindenbaum mixture. The distribution of activity in the various by-products, as well as the DDT, is shown in the table.

DISTRIBUTION OF C^{14} IN THE PRODUCTS OF THE DDT SYNTHESIS

Sample	A	B	C	D	E	F	G	H	I	J	K	L	M	DDT
Distribution, %	1.13	0.79	1.51	18.50	9.51	16.66	0.72	9.18	1.09	10.91	22.16	0.43	1.08	4.76

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Contents